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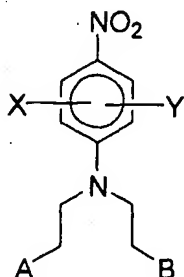
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(54) Title: NITROANILINE-BASED ALKYLATING AGENTS AND THEIR USE AS PRODRUGS



(I)

(57) Abstract: Nitroaniline-based unsymmetrical mustards of the general formula (I) are provided, together with methods of preparation and methods for their use as prodrugs for gene-dependent enzyme prodrug therapy (GDEPT) and cell ablation therapy in conjunction with nitroreductase enzymes as hypoxia selective cytotoxins and as anticancer agents.

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NITROANILINE-BASED ALKYLATING AGENTS
AND THEIR USE AS PRODRUGS

The present invention relates to the preparation of nitroaniline-based unsymmetrical
5 mustards, and their use as prodrugs for GDEPT (gene-dependent enzyme-prodrug therapy)
and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective
cytotoxins, and as anticancer agents.

BACKGROUND TO THE INVENTION

10 The use of tumour-selective prodrugs (relatively inactive compounds that can be
selectively converted to more active compounds *in vivo*) is a valuable concept in cancer
therapy.

15 For example a prodrug may be converted into an anti-tumour agent under the influence
of an enzyme that is linkable to a monoclonal antibody that will bind to a tumour
associated antigen. The combination of such a prodrug with such an enzyme
monoclonal/antibody conjugate represents a very powerful clinical agent. This
approach to cancer therapy, often referred to as "antibody directed enzyme/prodrug
20 therapy" (ADEPT), is disclosed in W088/07378.

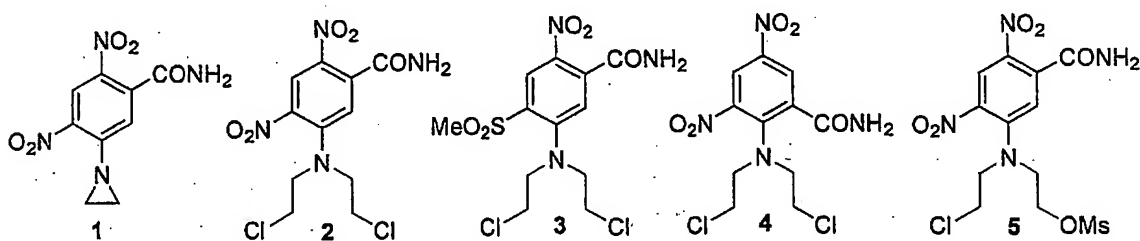
A further therapeutic approach termed "virus-directed enzyme prodrug therapy"
(VDEPT) has been proposed as a method for treating tumour cells in patients using
prodrugs. Tumour cells are targeted with a viral vector carrying a gene encoding an
25 enzyme capable of activating a prodrug. The gene may be transcriptionally regulated
by tissue specific promoter or enhancer sequences. The viral vector enters tumour cells
and expresses the enzyme, in order that a prodrug is converted to an active drug within
the tumour cells (Huber et al., *Proc. Natl. Acad. Sci. USA* (1991) 88, 8039).

Alternatively, non-viral methods for the delivery of genes have been used. Such
30 methods include calcium phosphate co-precipitation, microinjection, liposomes, direct
DNA uptake, and receptor-mediated DNA transfer. These are reviewed in Morgan &
French, *Annu. Rev. Biochem.*, 1993, 62; 191. The term "GDEPT" (gene-directed
enzyme prodrug therapy) is used to include both viral and non-viral delivery systems.

4-Nitroaromatic compounds are reduced by both mammalian and bacterial flavoprotein enzymes, which effect stepwise addition of up to six electrons. The major enzymic metabolite is usually the 4-electron species (hydroxylamine).

5 The present invention relates to novel nitroaniline-based unsymmetrical mustards having cytotoxic activity, to methods of preparing the novel compounds, and to the use of these compounds as prodrugs for GDEPT and for cell ablation therapy in conjunction with nitroreductase enzymes (particularly the nitro reductases encoded by the *nfsB* gene of
10 *E. coli* or by *Clostridia* species), as hypoxia-selective cytotoxins, and as anticancer agents.

Both dinitrobenzamide aziridines (e.g., 1) [Knox et al., *Cancer Met. Rev.*, 1993, 12, 195] and nitro- and dinitrobenzamide mustards (e.g., 2-4) [Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270] have been reported as substrates for the aerobic *E. coli* nitroreductase (NTR),
15 and as specific prodrugs for GDEPT in conjunction with NTR.

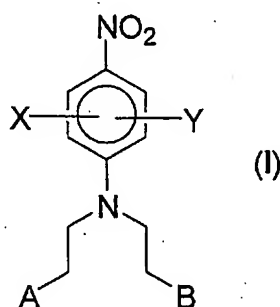


20 Unsymmetrical (chloro-mesylate) mustards have been reported [e.g., Marais et al., *Cancer Res.* 1996, 56, 4735], including the dinitro analogue 5 [Friedlos et al., *J. Med. Chem.* 1997, 40, 1270], which was described as not sufficiently potent for a full biological evaluation to be conducted.

25 It is therefore an object of the invention to provide a series of unsymmetrical mustards, methods for preparing the unsymmetrical mustards that are suitable for use as prodrugs for GDEPT (gene-dependent enzyme-prodrug therapy) and cell ablation therapy in conjunction with nitroreductase enzymes, as hypoxia-selective cytotoxins, and as
30 anticancer agents or to at least provide the public with a useful alternative.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a nitroaniline-based unsymmetrical
 5 mustard represented by the general formula (I);



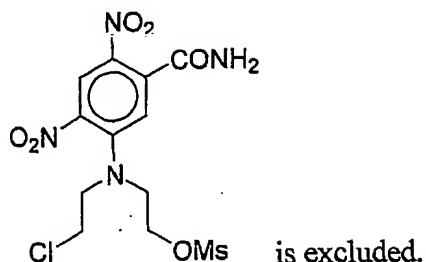
wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -
 10 lower alkyl optionally substituted with one or more hydroxy and/or one or more amino
 groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the
 tertiary amine is further included;

Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, $\text{CONR}^2\text{morpholide}$,
 CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$
 15 wherein each R^2 and R^3 independently represent a H, C_{1-6} -lower alkyl optionally
 substituted with one or more hydroxy and/or one or more amino groups; and
 A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or
 $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl
 optionally substituted with one or more hydroxy and/or one or more amino groups and
 20 wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide
 derivative of the tertiary amine is further included;

and pharmaceutically acceptable derivatives and salts thereof;

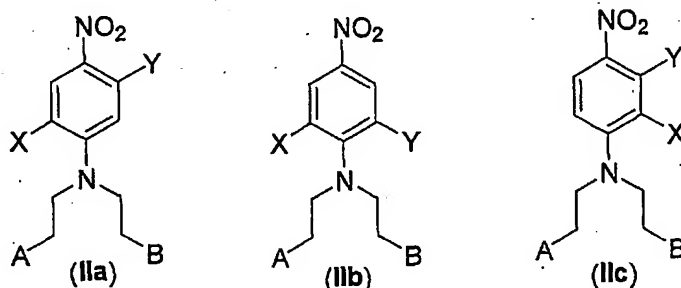
with the proviso

- (i) that $\text{A} \neq \text{B}$ and
- 25 (ii) that



In a preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (IIa-IIIc)

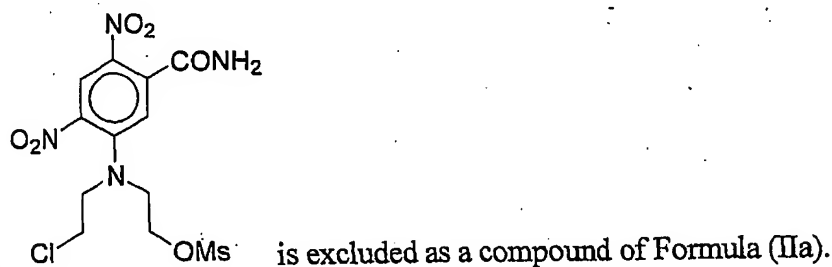
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wherein X, Y, A and B are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

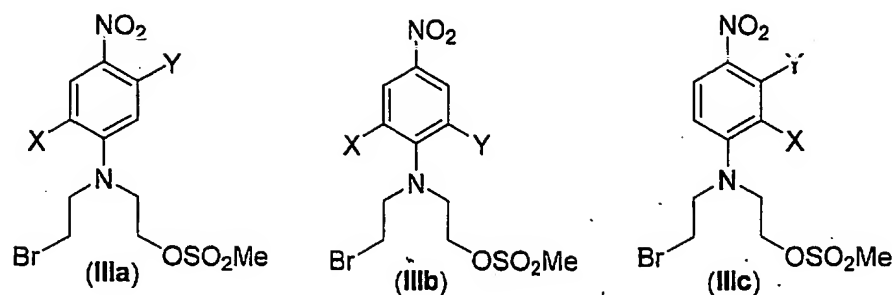
10 with the proviso

- (i) that $A \neq B$ and
- (iii) that



15 In a more preferred embodiment the nitroaniline-based unsymmetrical mustard is selected from a compound represented by one of formulae (IIIa-IIIc)

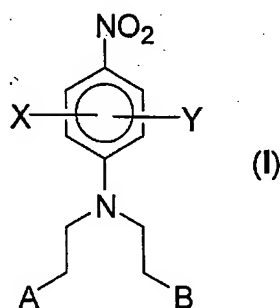
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wherein X , Y , are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof.

5

In a second aspect of the invention there is provided a method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



10

wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

15 Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, CONR^2 morpholide, CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$ wherein each R^2 and R^3 independently represent a H , C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

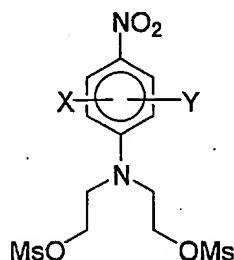
A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or
 20 $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

and pharmaceutically acceptable derivatives and salts thereof;
with the proviso

- (i) that $A \neq B$
the method including the step of

5

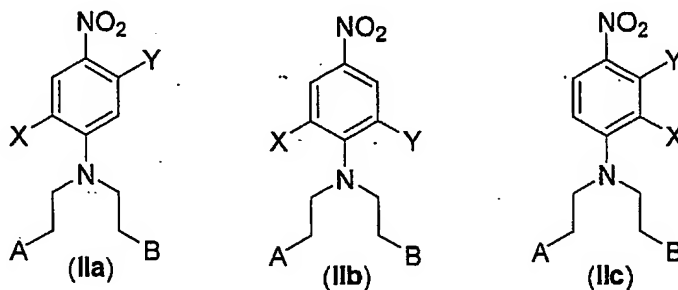
- (i) reacting a compound of



with an amount of an alkali metal halide in a polar solvent to give an
unsymmetrical halo-mesylate compound.

10

In a preferred embodiment the method of preparing a nitroaniline-based unsymmetrical
mustard represented by the general formula represented by one of formulae (IIa-IIc)



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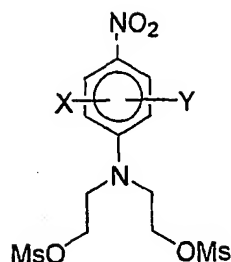
wherein X, Y, A and B are as defined above for a compound of Formula (I); and
pharmaceutically acceptable derivatives and salts thereof;

with the proviso

- (i) that $A \neq B$ and
the method including the step of

- (i) reacting a compound of

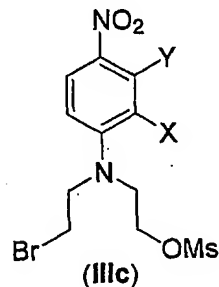
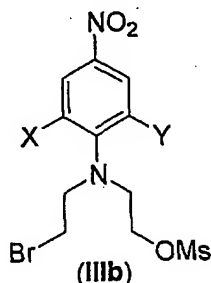
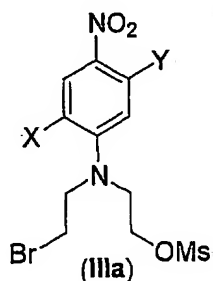
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with an amount of an alkali metal halide or mesylate halide in a polar solvent to give a unsymmetrical halo-mesylate compound.

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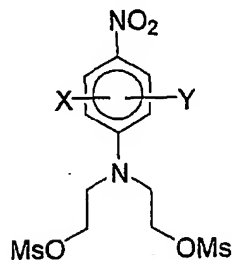
In a more preferred embodiment the method of preparing a nitroaniline-based unsymmetrical mustard represented by one of formulae (IIIa-IIIc)



10

wherein X, Y, are as defined above for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof; the method including the step of

(ii) reacting a compound of



15

with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (IIIa-IIIc).

It is preferred in the methods defined above that the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.

- 5 It is preferred in the methods defined above that the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI and NaBr.

In a third aspect there is provided a compound of formula (I) obtained by any one of the preparative methods defined above.

10

In a fourth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as hypoxia-selective cytotoxins, including the step of administering a compound of Formula I as defined above or a compound of Formulae
15 Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a “therapeutically effective amount” to tumour cells in a subject.

Preferably, the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E.Coli* or by *Clostridia* species.

20

In a fifth aspect, the present invention provides a method for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent including the step of administering a compound of Formula I as defined above or a compound of Formulae
25 Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a “therapeutically effective amount” to target tumour cells in a subject.

Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E.Coli* or by *Clostridia* species.

30

In a sixth aspect of the present invention, there is provided a method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as defined above or a compound of Formulae

Ia-Ic, IIa – IIc and IIIa-c as defined above or a mixture thereof in a “therapeutically effective amount” to ablate tumour cells in tissue in a subject, wherein said tissue expresses the at least one nitroreductase enzyme.

- 5 Preferably the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E. Coli* or by *Clostridia* species.

Preferably, the cell ablation therapy provides a substantially minimal bystander effect.

- 10 In a seventh aspect of the present invention there is provided a pharmaceutical composition including a therapeutically effective amount of a compound of formula I or a compound of formulae Ia-c, IIa-c, IIIa-c or a mixture thereof, and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.

- 15 The pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser should preferably be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, such as cutaneous, subcutaneous, or intravenous. It is to be appreciated that these factors could be readily determined by
20 someone skilled in the art without undue experimentation.

- Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water,
25 petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such as gelatin.

- 30 For intravenous, cutaneous or subcutaneous injection, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has a suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium

Chloride injection, Ringer's injection, Lactated Ringer's injection. Preservatives, stabilisers, buffers antioxidants and/or other additives may be included as required.

5 In an eighth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above, for use in GDEPT to target cancer cells in a subject in need thereof.

10 In a ninth aspect of the present invention there is provided, the use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined above or a compound of Formulae Ia-Ic, IIa – IIc and IIIa-c as defined above, for use in cell ablation therapy to target cancer cells in a subject in need thereof.

15 While the compounds of the present invention will typically be used to target tumour cells or tumour tissues in human subjects, they may be used to target tumour cells or tissues in other warm blooded animal subjects such as other primates, farm animals such as cattle, and sports animals and pets such as horses, dogs, and cats.

20 As used throughout the specification the term "therapeutically effective amount", is to be understood as an amount of a compound of Formula I as defined above or a compound of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof that is sufficient to show benefit to a subject with cancer cells. The actual amount, rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment is within the responsibility of general practitioners and
25 other medical doctors.

It is to be understood that the compounds of the invention as defined above may be administered alone or in combination with other treatments, especially radiotherapy, either simultaneously or sequentially dependent upon the condition to be treated.

30

As used throughout the specification the pharmaceutically acceptable derivatives and salts thereof include acid derived salts formed from are hydrochloric, sulfuric, phosphoric, acetic, citric, oxalic, malonic, salicylic, malic, fumaric, succinic, ascorbic, maleic,

methanesulfonic, isethionic acids and the like and base derived salts formed from sodium and potassium carbonate, sodium and potassium hydroxide, ammonia, triethylamine, triethanolamine and the like.

- 5 The technique of cell ablation therapy, would be known to someone skilled in the art. This therapy can be used to selectively ablate specified target cells or tissue through specific enzymatic expression of a nitroreductase for example, that is specifically expressed by the tissue and which can then be employed to active a prodrug into an active metabolite to ablate the specified target cells or tissue. (Gusterson *et al. Endocrine Related Cancer*,
10 1997, 4, 67-74.)

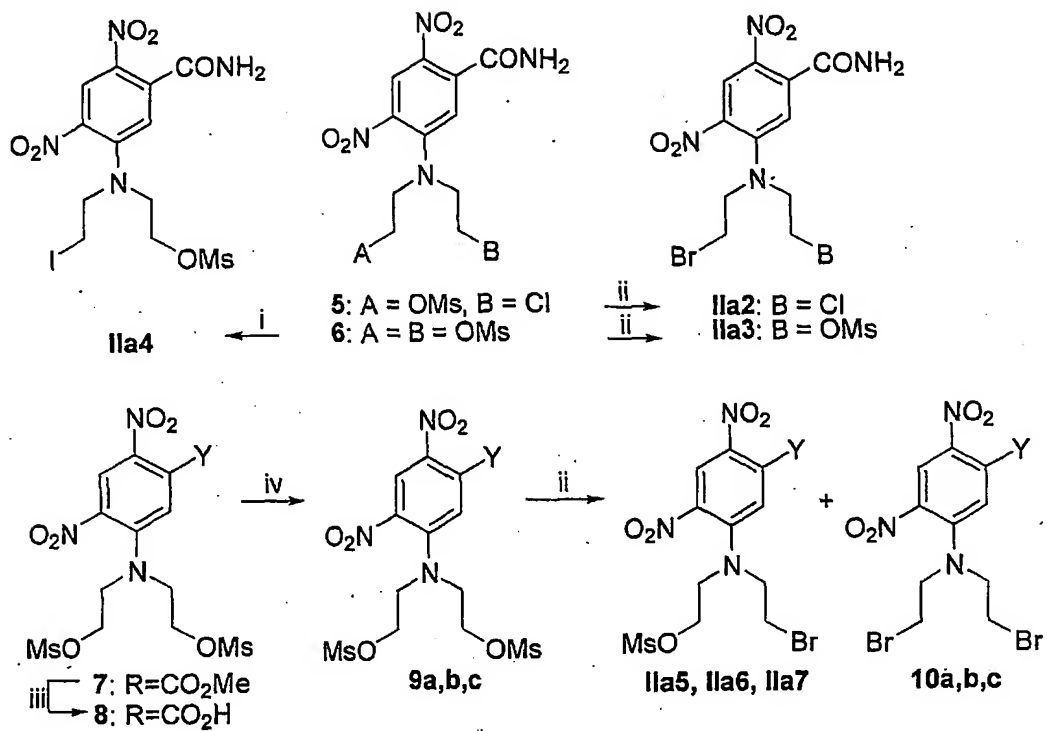
- The expression "substantially minimal bystander effect" is to be understood as meaning that the killing of adjoining non-targeted tumour cells is minimal as a result of diffusion between the targeted tumour cells and non-targeted tumour cells of an activated metabolite
15 that arises from the enzymatic activation of a compound of Formula I as defined above or a compound of any one of compounds Ia-c, IIa-c and IIIa-c as defined above or a mixture thereof.

- Further aspects of the present invention will become apparent from the following
20 description given by way of example only and with reference to the accompanying synthetic schemes.

DETAILED DESCRIPTION OF THE INVENTION

- 25 The compounds of formula (I) and the acid addition salts and N-oxides thereof may be prepared by the processes outlined in Schemes 1-3, examples of which are found in Examples A-C.

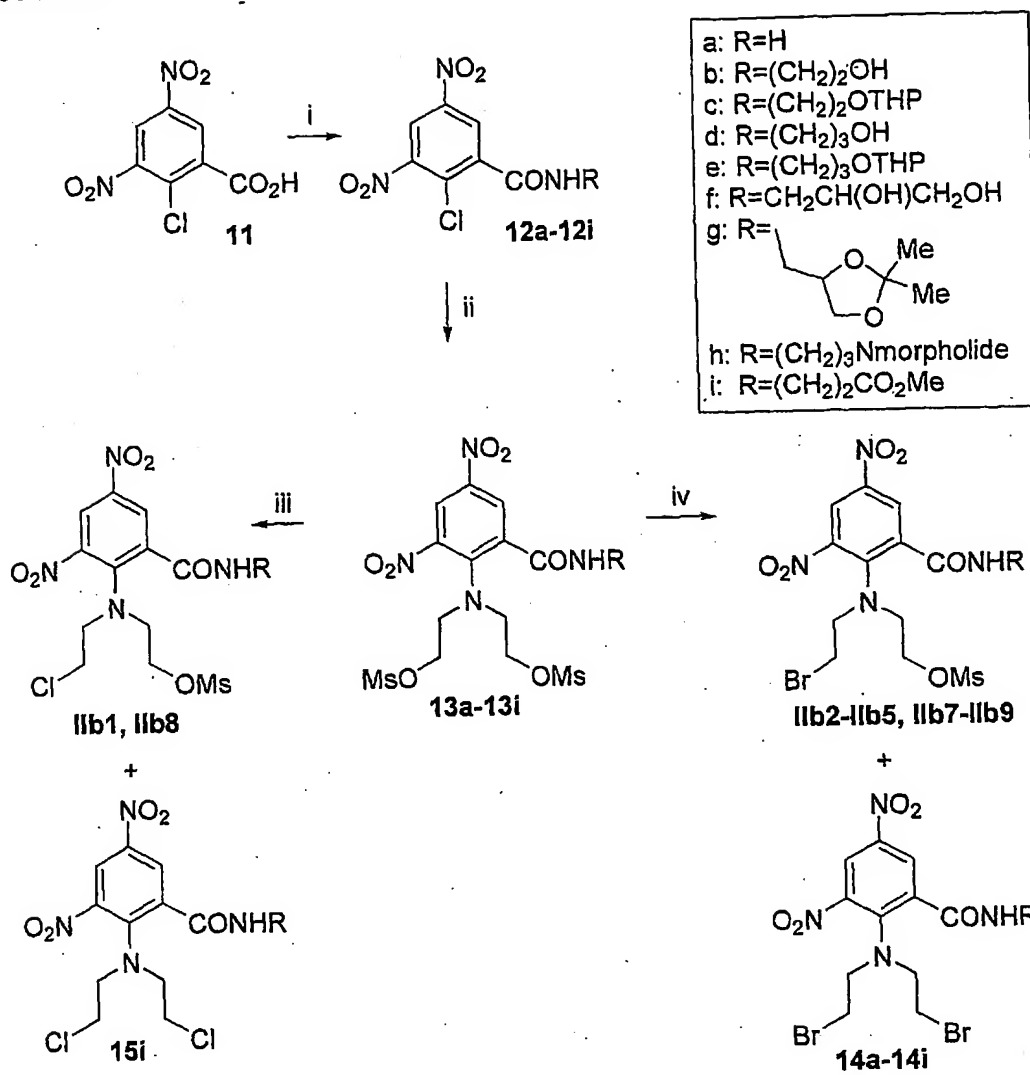
Scheme 1.



Reagents:
 (i) NaI/DMF;
 (ii) LiBr/MeCN;
 (iii) KOH;
 (iv) SOCl₂, then RNH₂.

a: Y = (CH₂)₂OH
 b: Y = (CH₂)₃OH
 c: Y = CH₂CH(OH)CH₂OH

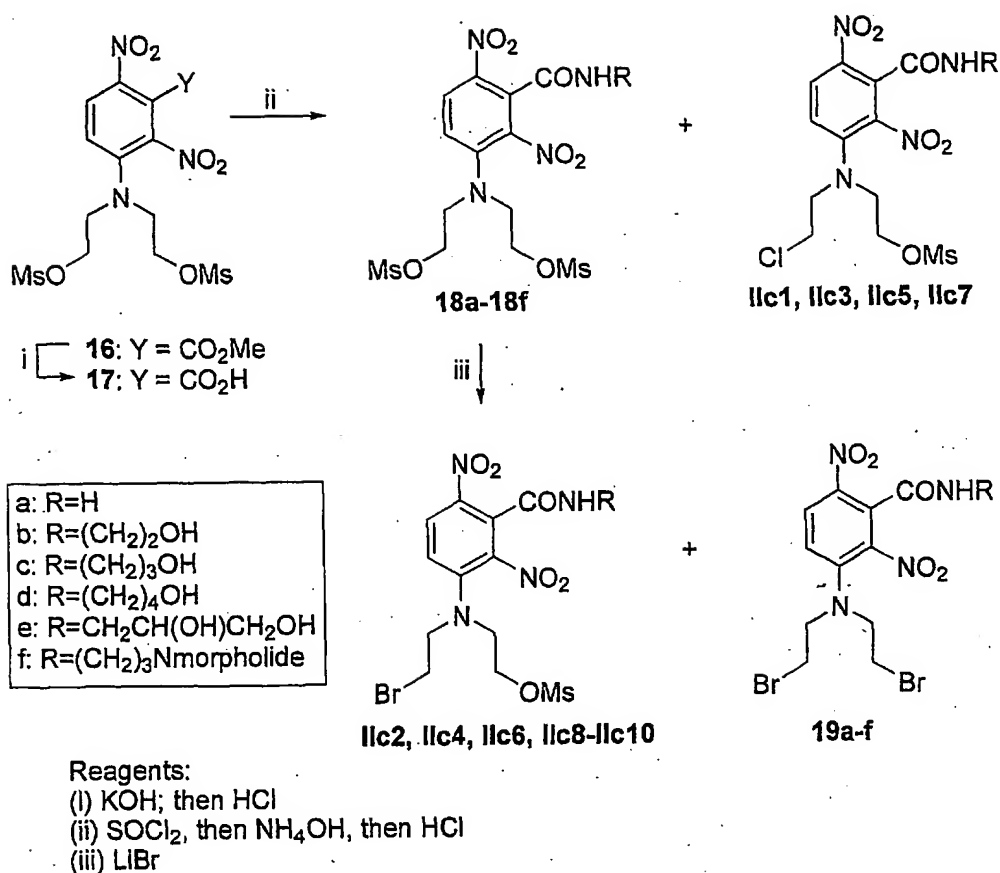
Scheme 2



Reagents:

- (i) RNH₂;
 (ii) HN(CH₂CH₂OH)₂, then MsCl;
 (iii) LiCl;
 (iv) LiBr.

Scheme 3.



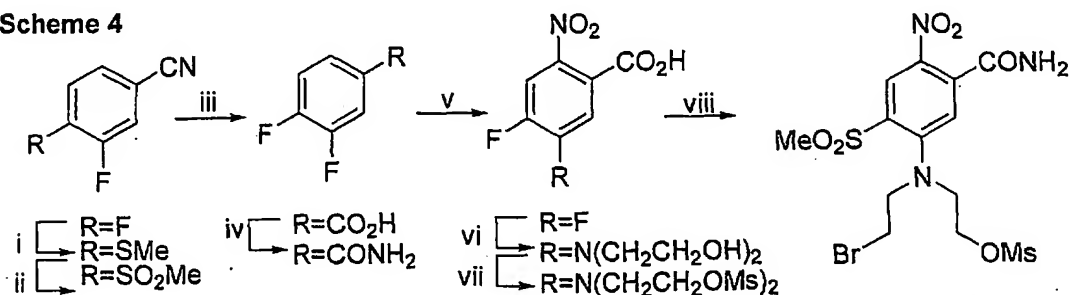
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In Schemes 1-3, the key reaction is reaction of the dimesylates **6**, **9**, **13a-13g** and **18a-18d** with strictly controlled amounts of LiBr or NaI in a polar solvent like DMF or MeCN to give the unsymmetrical bromo- and iodo-mesylate mustards. The method can also be adapted to reaction of the known chloromesylate (**5**) to give the unsymmetrical chloro/bromo mustard **11a2**. While this reaction gives varying amounts of the corresponding bis(bromo) or bis(iodo) compounds as well, these can be easily separated by chromatography to give the pure unsymmetrical mustards.

Compounds of formula I wherein X represents SO₂Me, Y represents CONR²R³, and A and B each independently represent halogen or OSO₂R⁴ (with the proviso that A ≠ B) can be prepared by the general route outlined (for a specific example) in Scheme 4, from 3,4-difluorobenzonitrile [after Atwell et al., *Anti-Cancer Drug Design*, 1996, 11, 553-567]. Reaction of this with NaSMe followed by oxidation provides the SO₂Me

group, and the nitrile is then elaborated to the CONR^2R^3 function. Displacement of the 4-F group with diethanolamine, followed by elaboration, gives the required asymmetric mustards.

Scheme 4



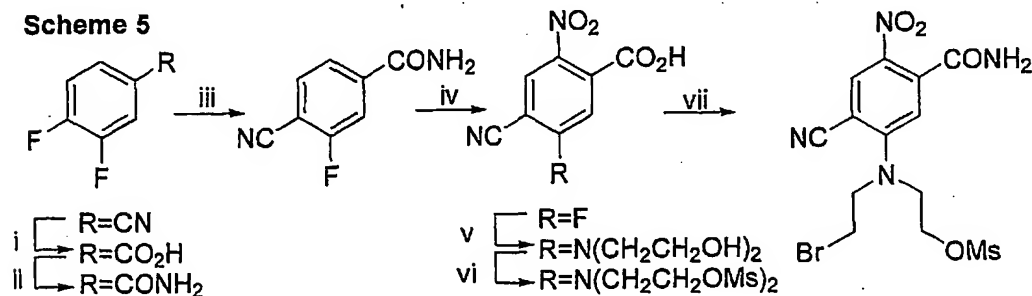
(i) NaSMe; (ii) NaBO₃; (iii) H₂SO₄/AcOH; (iv) SOCl₂, then NH₄OH; (v) HNO₃/H₂SO₄; (vi) HN(CH₂CH₂OH)₂/DMSO; (vii) MsCl; (viii) LiBr/DMF.

5

Compounds of formula I wherein X represents CN, Y represents CONR^2R^3 , and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $\text{A} \neq \text{B}$) can be prepared by the general route outlined (as shown for a specific example) in Scheme 5, from 3,4-difluorobenzonitrile [after Atwell et al., *Anti-Cancer Drug Design*, 1996, 11, 553-567]. Conversion of the nitrile to a carboxamide (hydrolysis followed by amination), then displacement of the 3-F with TMS-CN, followed by reaction of the 4-F group with diethanolamine and subsequent elaboration as in Scheme 4 gives the required asymmetric mustard.

15

Scheme 5



ii) H₂SO₄/AcOH; (ii) SOCl₂, then NH₄OH; (iii) TMS-CN; (iv) HNO₃/H₂SO₄; (v) HN(CH₂CH₂OH)₂/DMSO; (vi) MsCl; (vii) LiBr/DMF.

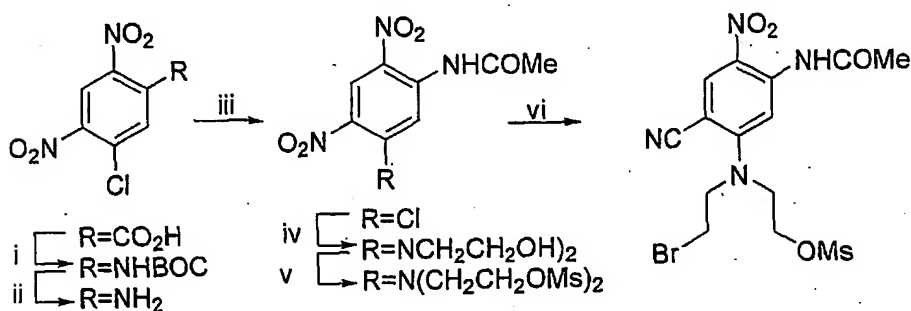
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Compounds of formula I wherein X represents NO₂, Y represents NHCOR^2 , and A and B each independently represent halogen or OSO_2R^4 (with the proviso that $\text{A} \neq \text{B}$) can be prepared by the general route outlined (as shown for a specific example) in Scheme

6, from 2,4-dinitro-5-chlorobenzoic acid. Curtius reaction with DPPA, followed by hydrolysis and acetylation gives the acetamide. Reaction of the 5-Cl group with diethanolamine and subsequent elaboration as in Scheme 4 gives the required asymmetric mustard.

5

Scheme 6

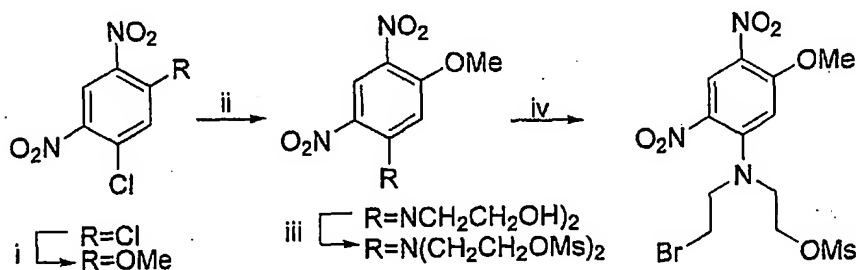


ii) DPPA/t-BuOH; (ii) TFA; (iii) Ac₂O/pyridine; (iv) HN(CH₂CH₂OH)₂/DMSO; (v) MsCl; (vi) LiBr/DMF

Compounds of formula I wherein X represents NO₂, Y represents OR², and A and B each independently represent halogen or OSO₂R⁴ (with the proviso that A ≠ B) can be prepared by the general route outlined (as shown for a specific example) in Scheme 7, from 1,5-dichloro-2,4-dinitrobenzene. Reaction of the more active 1-Cl group with NaOMe gives the methyl ether, and subsequent elaboration of the 5-Cl group as in Scheme 4 gives the required asymmetric mustard.

10

Scheme 7



15

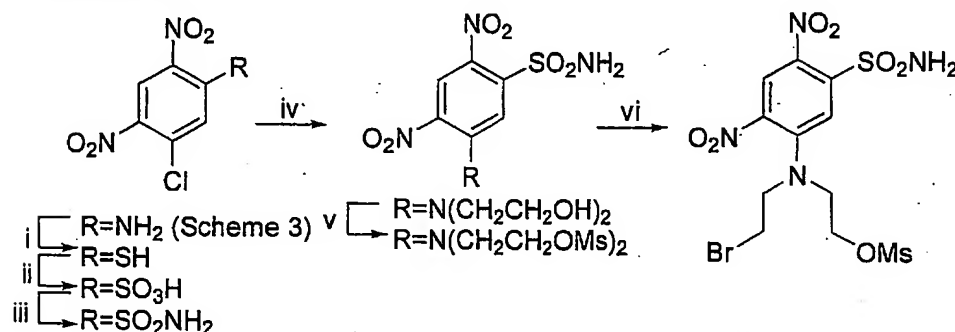
ii) Na/MeOH; (ii) HN(CH₂CH₂OH)₂/DMSO; (iii) MsCl; (iv) LiBr/DMF

Compounds of formula I wherein X represents NO₂, Y represents SO₂NHR², and A and B each independently represent halogen or OSO₂R⁴ (with the proviso that A ≠ B) can be prepared by the general route outlined (as shown for a specific example) in Scheme

8, from 5-chloro-2,4-dinitroaniline (see Scheme 6). Diazotization followed by oxidation and amination provides the sulphonamide [Herbert RB & Hollman RG. *Tetrahedron* 1965, 21, 663-675], and subsequent elaboration of the 5-Cl group as in Scheme 4 gives the required asymmetric mustard.

5

Scheme 8



(i) diazotize, then H_2S ; (ii) oxone; (iii) SOCl_2 , then NH_4OH ; (iv) $\text{HN}(\text{CH}_2\text{CH}_2\text{OH})_2/\text{dioxane}$; (v) MsCl ; (vi) LiBr/DMF

The following Table 1 sets out physicochemical data for 25 compounds within the general formula I, representative of it, and preparable by the processes of the invention.

10

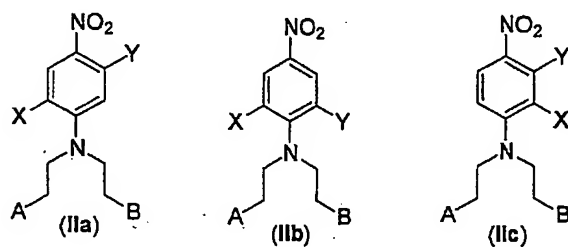


Table 1

| No | Y | X | A | B | PRIOR ART COMPOUND | | |
|--------------------------------|-------------------|-----------------|----|-----|---|---|-------------|
| 5 | CONH ₂ | NO ₂ | Cl | OMs | [Friedlos et al., <i>J. Med Chem.</i> 1997, 40, 1270] | | |
| <i>Examples of formula IIa</i> | | | | | | | |
| No | Y | X | A | B | mp (°C) | formula | analyses |
| IIa2 | CONH ₂ | NO ₂ | Cl | Br | 153 | C ₁₁ H ₁₂ BrClN ₄ O ₅ | C, H, N, Cl |
| IIa3 | CONH ₂ | NO ₂ | Br | OMs | 160-161 | C ₁₂ H ₁₅ BrN ₄ O ₈ S | C, H, N, Br |
| IIa4 | CONH ₂ | NO ₂ | I | OMs | 160 | C ₁₂ H ₁₅ IN ₄ O ₈ S | C, H, N, I |

| | | | | | | | |
|--------------------------------|--|-----------------|----|-----|---------|---|-------------|
| IIa5 | CONH(CH ₂) ₂ OH | NO ₂ | Br | OMs | 102-104 | C ₁₄ H ₁₉ Br ₄ N ₄ O ₉ S | C, H, N, Br |
| IIa6 | CONH(CH ₂) ₃ OH | NO ₂ | Br | OMs | gum | C ₁₅ H ₂₁ Br ₄ N ₄ O ₉ S | HRMS |
| IIa7 | CONHCH ₂ CH(OH)- CH ₂ OH | NO ₂ | Br | OMs | 117-118 | C ₁₅ H ₂₁ BrN ₄ O ₁₀ S | C, H, N, Cl |
| <i>Examples of formula IIb</i> | | | | | | | |
| No | Y | X | A | B | mp (°C) | formula | analyses |
| IIb1 | CONH ₂ | NO ₂ | Cl | OMs | 155-157 | C ₁₂ H ₁₅ ClN ₄ O ₈ S | C, H, N, Cl |
| IIb2 | CONH ₂ | NO ₂ | Br | OMs | 153-154 | C ₁₂ H ₁₅ BrN ₄ O ₈ S | C, H, N, Br |
| IIb3 | CONH(CH ₂) ₂ OH | NO ₂ | Br | OMs | gum | C ₁₄ H ₁₉ BrN ₄ O ₉ S | HRMS |
| IIb4 | CONH(CH ₂) ₂ OH | NO ₂ | I | OMs | gum | C ₁₄ H ₁₉ IN ₄ O ₉ S | |
| IIb5 | CONH(CH ₂) ₃ OH | NO ₂ | Br | OMs | oil | C ₁₅ H ₂₁ BrN ₄ O ₉ S | |
| IIb6 | CONHCH ₂ CH(OH)- CH ₂ OH | NO ₂ | Br | OMs | gum | C ₁₅ H ₂₁ BrN ₄ O ₁₀ S | C, H, N, Br |
| IIb7 | CONH(CH ₂) ₃ Nmorph | NO ₂ | Br | OMs | gum | C ₁₉ H ₂₈ BrN ₅ O ₉ S | HRMS |
| IIb8 | CONH(CH ₂) ₂ CO ₂ Me | NO ₂ | Cl | OMs | oil | C ₁₆ H ₂₁ ClN ₄ O ₁₀ S | HRMS |
| IIb9 | CONH(CH ₂) ₂ CO ₂ Me | NO ₂ | Br | OMs | gum | C ₁₆ H ₂₁ BrN ₄ O ₁₀ S | HRMS |
| <i>Examples of formula IIc</i> | | | | | | | |
| No | Y | X | A | B | mp (°C) | formula | analyses |
| IIc1 | CONH ₂ | NO ₂ | Cl | OMs | 134-136 | C ₁₂ H ₁₅ ClN ₄ O ₈ S | C, H, N, S |
| IIc2 | CONH ₂ | NO ₂ | Br | OMs | 143-145 | C ₁₂ H ₁₅ BrN ₄ O ₈ S | C, H, N, Br |
| IIc3 | CONH(CH ₂) ₂ OH | NO ₂ | Br | OMs | 94-97 | C ₁₄ H ₁₉ BrN ₄ O ₉ S | C, H, N |
| IIc4 | CONH(CH ₂) ₃ OH | NO ₂ | Cl | OMs | 104-109 | C ₁₅ H ₂₁ ClN ₄ O ₉ S | C, H, N, Cl |
| IIc5 | CONH(CH ₂) ₃ OH | NO ₂ | Br | OMs | 115-117 | C ₁₅ H ₂₁ BrN ₄ O ₉ S | C, H, N |
| IIc6 | CONH(CH ₂) ₄ OH | NO ₂ | Br | OMs | 114-117 | C ₁₆ H ₂₃ BrN ₄ O ₉ S | C, H, N |
| IIc7 | CONHCH ₂ CH(OH)- CH ₂ OH | NO ₂ | Cl | OMs | 100-105 | C ₁₅ H ₂₁ ClN ₄ O ₁₀ S | C, H, N, Cl |
| IIc8 | CONH ₂ CH ₂ CH(OH)C H ₂ OH | NO ₂ | Br | OMs | 108-110 | C ₁₅ H ₂₁ BrN ₄ O ₁₀ S | C, H, N, Br |
| IIc9 | CONH(CH ₂) ₃ Nmorph | NO ₂ | Cl | OMs | 113-116 | C ₁₉ H ₂₈ ClN ₅ O ₉ S | HRMS |
| IIc10 | CONH(CH ₂) ₃ Nmorph | NO ₂ | Br | OMs | 114-117 | C ₁₉ H ₂₈ BrN ₅ O ₉ S | HRMS |

The following Examples A-C illustrate the preparation of compounds representative of the general formula (I).

Example A : Preparation of analogues of class IIa by the method outlined in Scheme 1.

5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide (IIa2). A mixture of
2-[5-(aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (5)
[Friedlos et al., *J. Med. Chem.* 1997, 40, 1270] (0.91 g, 2.2 mmol) and LiBr (0.21 g, 2.4
mmol) in anhydrous MeCN (25 mL) was stirred under reflux for 1.5 h, then
concentrated under reduced pressure. The residue was chromatographed on silica gel,
eluting with CH₂Cl₂/EtOAc (3:2) to give a crude product contaminated with the
corresponding dibromo mustard. Purification by multiple recrystallisations from
EtOAc/I-Pr₂O gave **IIa2** (595 mg, 68%): mp 153 °C; ¹H NMR [(CD₃)₂SO] δ 8.52 (s, 1
H, H-3), 8.17 & 7.82 (2 x s, 2 H, CONH₂), 7.43 (s, 1 H, H-6), 3.82 (t, *J* = 5.8 Hz, 2 H,
CH₂Cl), 3.77-3.63
(m, 6 H, N(CH₂-)CH₂CH₂Br). Anal. Calc for C₁₁H₁₂BrClN₄O₅: C, 33.4; H, 3.1; N,
14.2; Cl, 9.6. Found: C, 33.4; H, 3.0; N, 14.1; Cl, 8.9%.

**2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate
(IIa3).** A mixture of 2-(5-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-
dinitroanilino)ethyl methanesulfonate (6) [Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270]
(1.60 g, 3.4 mmol) and LiBr (356 mg, 4.1 mmol) in anhydrous MeCN (30 mL) was
stirred under reflux for 1 h. The mixture was concentrated under reduced pressure and
the residue was chromatographed on silica gel. Elution with EtOAc/CH₂Cl₂ (11:9)
gave the dibromo mustard, while further elution with EtOAc/CH₂Cl₂ (3:1) gave **IIa3**
(0.61 g, 39%): mp (EtOAc/I-Pr₂O) 160-161 °C; ¹H NMR [(CD₃)₂SO] δ 8.53 (s, 1 H, H-
3), 8.14 & 7.83 (2 x s, 2 H, CONH₂), 7.46 (s, 1 H, H-6), 4.33 (t, *J* = 5.1 Hz, 2 H, CH₂O),
3.74 (t, *J* = 5.1 Hz, 2 H, CH₂CH₂O), 3.70 (br s, 4 H, CH₂CH₂Br), 3.14 (s, 3 H, CH₃).
Anal. Calcd for C₁₂H₁₅BrN₄O₈S: C, 31.7; H, 3.3; N, 12.3; Br, 17.6. Found: C, 32.0; H,
3.4; N, 12.2; Br, 17.7%.

2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIa4).
A mixture of **6** (1.12 g, 2.38 mmol) and NaI (0.46 g, 3.07 mmol) in anhydrous MeCN (20
mL) was stirred at reflux for 1 h. The mixture was concentrated under reduced pressure
and the residue was chromatographed on silica gel. Elution with EtOAc/CH₂Cl₂ (1:1)

gave the diiodo mustard, while further elution with EtOAc/CH₂Cl₂ (3:1) gave **IIa4** (0.49 g, 41%): mp (Me₂CO/EtOAc/I-Pr₂O) 160 °C; ¹H NMR [(CD₃)₂SO] δ 8.52 (s, 1 H, H-3), 8.14 & 7.83 (2 x s, 2 H, NH₂), 7.44 (s, 1 H, H-6), 4.33 (t, *J* = 5.1 Hz, 2 H, CH₂O), 3.73 (t, *J* = 5.1 Hz, 2 H, CH₂CH₂O), 3.65 (t, *J* = 6.9 Hz, 2 H, CH₂CH₂I), 3.40 (t, *J* = 6.9 Hz, 2 H, CH₂I), 3.13 (s, 3 H, CH₃). Anal. Calcd for C₁₂H₁₅IN₄O₈S: C, 28.7; H, 3.0; N, 11.2; I, 25.3. Found: C, 29.4; H, 3.0; N, 11.0; I, 25.0%.

2-((2-Bromoethyl)5-[(2-hydroxyethyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIa5). A stirred solution of methyl 5-[bis(2-hydroxyethyl)amino]-2,4-dinitrobenzoate [Palmer et al., *J. Med. Chem* 1994, 37, 2175] (5.50 g, 16.7 mmol) and Et₃N (5.82 mL, 41.8 mmol) in dry CH₂Cl₂ (50 mL) was treated dropwise at 0 °C with MsCl (3.14 mL, 40.0 mmol). After 30 min, 10% aqueous KHCO₃ (100 mL) was added, and the mixture was stirred for a further 30 min at 0 °C and then diluted with pet. ether (500 mL). The precipitated product was collected and washed with water and iPr₂O to give methyl 5-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoate (**7**) (7.44 g, 92%): mp (CH₂Cl₂/pet. ether) 157-158 °C; ¹H NMR [(CD₃)₂SO] δ 8.62 (s, 1 H, H-3), 7.77 (s, 1 H, H-6), 4.35 (t, *J* = 5.1 Hz, 4 H, 2xCH₂O), 3.88 (s, 3 H, CO₂CH₃), 3.73 (t, *J* = 5.1 Hz, 4 H, N(CH₂)CH₂), 3.13 (s, 6 H, 2xSO₂CH₃). Anal calcd for C₁₄H₁₉N₂O₁₂S₂: C, 34.6; H, 3.9; N, 8.7; S, 13.2. Found: C, 34.8; H, 3.7; N, 8.9; S, 13.1%.

Hydrolysis of **7** (3.0 g, 6.18 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at room temperature for 15 min followed by acidification with 1 N HCl and extraction with EtOAc gave a quantitative yield of 5-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid (**8**), mp 200-210 °C, which was used for the next step without further purification; ¹H NMR [(CD₃)₂SO] δ 14.1 (v br s, 1 H, CO₂H), 8.57 (s, 1 H, H-3), 7.69 (s, 1 H, H-6), 4.34 (t, *J* = 5.1 Hz, 4 H, 2xCH₂O), 3.72 (t, *J* = 5.1 Hz, 4 H, 2xCH₂CH₂O), 3.13 (s, 6 H, 2xCH₃).

A suspension of **8** (3.20 g, 6.79 mmol) in SOCl₂ (60 mL) containing DMF (2 drops) was heated under reflux for 1 h. Evaporation of the solvent under reduced pressure, followed by azeotroping in with benzene gave the crude acid chloride, which was dissolved in dry Me₂CO (80 mL) and treated at 0 °C with 2-aminoethanol (1.24 g, 20.3 mmol). After stirring at 0 °C for 5 min, the mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added. The mixture was extracted with EtOAc (2x) and the combined extracts were washed with saturated NaBr

solution, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica gel, eluting with EtOAc/MeOH (15:1) to give 2-(5-[(2-hydroxyethyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9a**) (2.87 g, 82%) as a gum that was used directly.

5

A mixture of **9a** (1.80 g, 3.50 mmol) and LiBr (0.43 g, 4.95 mmol) in DMF (5 mL) was stirred at 60 °C for 2 h. The reaction was then poured into saturated NaBr solution and extracted with EtOAc (2x). The combined extracts were washed with saturated NaBr solution, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was chromatographed on silica gel, eluting with EtOAc, to give 5-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,4-dinitrobenzamide (**10a**) (0.78 g, 46%): mp (MeOH/EtOAc/pet. ether) 151-152 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.73 (t, $J = 5.7$ Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.76 (t, $J = 5.6$ Hz, 1 H, OH), 3.77-3.64 (m, 8 H, $\text{N}(\text{CH}_2\text{CH}_2\text{Br})_2$), 3.53 (q, $J = 6.0$ Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, $J = 6.1$ Hz, 2 H, CONHCH_2). Anal. calcd for $\text{C}_{13}\text{H}_{16}\text{Br}_2\text{N}_4\text{O}_6$: C, 32.3; H, 3.3; N, 11.6; Br, 33.0. Found: C, 32.6; H, 3.3; N, 11.6; Br, 33.3%.

15

Further elution with EtOAc/MeOH (9:1) gave **IIa5** (0.73 g, 42%): mp (EtOAc) 102-104 °C; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.70 (t, $J = 5.7$ Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.76 ($J = 5.5$ Hz, 1 H, OH), 4.34 (t, $J = 5.1$ Hz, 2 H, CH_2OSO_2), 3.74 (t, $J = 5.1$ Hz, 2 H, $\text{CH}_2\text{CH}_2\text{OSO}_2$), 3.70 (br s, 4 H, $\text{CH}_2\text{CH}_2\text{Br}$), 3.53 (q, $J = 6.0$ Hz, 2 H, CH_2OH), 3.31 (q, partially obscured, $J = 6.1$ Hz, 2 H, CONHCH_2), 3.14 (s, 3 H, CH_3). Anal. calcd for $\text{C}_{14}\text{H}_{19}\text{BrN}_4\text{O}_9\text{S}$: C, 34.3; H, 3.9; N, 11.0; Br, 15.9. Found: C, 33.8; H, 3.8; N, 11.2; Br, 16.0%.

20

25 2-((2-Bromoethyl)5-[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate (**IIa6**). 5-(Bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,4-dinitrobenzoic acid (**8**) was heated under reflux in excess SOCl_2 (60 mL) and catalytic DMF for 1 h. Evaporation under reduced pressure, followed by azeotroping in benzene, gave the crude acid chloride. This was dissolved in dry Me_2CO and treated at 0 °C with 3-amino-1-propanol at 0 °C for 5 min. The mixture was acidified to pH 2-3 with 0.2 N HCl, concentrated to half volume, and then solid NaBr was added, followed by extraction with EtOAc (2x). Evaporation, and chromatography of the residue on silica gel, eluting with EtOAc/MeOH (9:1), gave 2-(5-[(3-

30

hydroxypropyl)amino]carbonyl}{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**9b**) (68%) as a yellow gum; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.54 (t, $J = 5.7$ Hz, 1 H), 8.53 (s, 1 H), 7.45 (s, 1 H), 4.43 (t, $J = 5.1$ Hz, 1 H), 4.33 (t, $J = 5.2$ Hz, 4 H), 3.69 (t, $J = 5.2$ Hz, 4 H), 3.57 (q, $J = 5.9$ Hz, 2 H), 3.26 (after D_2O exchange, t, $J = 7.0$ Hz, 1 H), 3.12 (s, 6 H), 1.66 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_{12}\text{S}$ (MH^+) m/z 529.0910; found 529.0904.

A solution of **9b** in DMF was treated with LiBr (1.4 equiv.), and worked up as above, and the product was chromatographed on silica gel. Elution with EtOAc gave a small amount of the dibromo mustard **10b**, while elution with EtOAc/MeOH (19:1) gave **IIa6** (31%) as a yellow gum: ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.60 (t, $J = 5.6$ Hz, 1 H), 8.54 (s, 1 H), 7.44 (s, 1 H), 4.45 (t, $J = 5.2$ Hz, 1 H), 4.33 (t, $J = 5.1$ Hz, 2 H), 3.74 (t, $J = 5.2$ Hz, 2 H), 3.72-3.66 (m, 4 H), 3.49 (q, $J = 5.9$ Hz, 2 H), 3.27 (after D_2O exchange, t, $J = 7.0$ Hz, 2 H), 3.14 (s, 3 H), 1.68 (pent, $J = 6.7$ Hz, 2 H). HRMS (FAB) calcd. for $\text{C}_{15}\text{H}_{22}^{79}\text{BrN}_4\text{O}_9\text{S}$ (MH^+) m/z 515.0270; found 515.0283.

2-((2-Bromoethyl)-5-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIa7). Reaction of the crude acid chloride made as above from acid **8** (2.9 g, 6.15 mmol) was dissolved in Me_2CO (100 mL), cooled in an ice-bath and treated with an excess of 3-amino-1,2-propanediol. After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, most of the solvent was evaporated, and the residue was partitioned between water and EtOAc. The aqueous layer was re-extracted with EtOAc and the combined organic phases were dried and evaporated. The residue was adsorbed directly onto silica gel and chromatographed, elution with EtOAc/MeOH (from 50:1 to 10:1) giving **2-(5-[(2,3-dihydroxypropyl)amino]carbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (9c)** (2.92 g, 87%) as a yellow oil; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.66 (t, $J = 5.8$ Hz, 1 H, CONH), 8.54 (s, 1 H, H-3), 7.48 (s, 1 H, H-6), 4.81 (d, $J = 5.0$ Hz, 1 H, CHO H), 4.59 (t, $J = 5.1$ Hz, 1 H, CH $_2\text{OH}$), 4.35 (m, 4 H, 2x CH $_2\text{OMs}$), 3.66 (m, 4 H), 3.62 (m, 1 H), 3.46-3.36 (m, 4 H), 3.13 (s, 6 H); ^{13}C NMR δ 164.48, 147.09, 138.26, 137.27, 136.60, 124.17, 121.72, 70.02, 66.69, 63.68, 50.21, 42.68, 36.55. HRMS m/z ($\text{M}+1$) $^+$ required for $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_{13}\text{S}_2$ 545.08596; Found 545.0856.

A solution of **9c** (1.28 g, 2.53 mmol) was dissolved in EtOAc (100 mL) and treated with LiBr (347 mg, 4.0 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 1:0 to 10:2) gave 5-[bis(2-bromoethyl)amino]-*N*-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide (**10c**) (0.4 g, 31%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.71 (t, *J* = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.43 (s, 1 H, H-6), 4.86 (d, *J* = 5.0 Hz, 1 H, CHOH), 4.59 (t, *J* = 5.8 Hz, 1 H, CH₂OH), 3.70 – 3.10 (m, 13 H); ¹³C NMR δ 164.61, 146.65, 137.99, 137.35, 136.52, 124.25, 121.20, 70.05, 63.73, 52.44, 42.76, 30.33. HRMS *m/z* (*M*+1)⁺ required for C₁₄H₁₉⁷⁹Br₂N₄O₇ 512.9621; Found 512.9596.

Further elution gave **IIa7** (0.62 g, 46%): mp (EtOAc) 117-118 °C; ¹H NMR [(CD₃)₂SO] δ 8.68 (t, *J* = 5.8 Hz, 1 H, CONH), 8.53 (s, 1 H, H-3), 7.46 (s, 1 H, H-6), 4.82 (d, *J* = 5.0 Hz, 1 H, CHOH), 4.56 (t, *J* = 5.1, 1 H, CH₂OH), 4.32 (m, 2 H, CH₂OMs), 3.75-3.60 (m, 7 H), 3.46-3.36 (m, 4 H), 3.13 (s, 3 H); ¹³C NMR δ 164.48, 146.84, 138.05, 137.29, 136.52, 124.18, 121.40, 70.01, 66.74, 63.68, 52.89, 49.56, 42.69, 36.55, 30.20. Anal. Calcd for C₁₅H₂₁BrN₄O₁₀S: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%.

Further elution gave starting material (**9c**) (0.27 g, 20%).

20 Example B: Preparation of analogues of class IIb by the method outlined in Scheme 2.

2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate (IIb1). A solution of 2-[bis(2-hydroxyethyl)amino]-3,5-dinitrobenzamide [Friedlos et al., *J. Med. Chem.*, 1997, 40, 1270] (2.5 g, 8 mmol) in CH₂Cl₂ (200 mL) was cooled in an ice-bath and Et₃N (8 mL) and MsCl (4 mL) were added in one portion. After stirred for 10 min, satd. NaHCO₃ (100 mL) was added, and after a further 30 min the aqueous phase was extracted with CH₂Cl₂ (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0), gave **IIb1** (0.6 g, 18%): mp (EtOAc/petroleum ether) 155-157 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.34 (d, *J* = 2.7 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH), 4.29 (m, 2 H, CH₂OMs), 3.73 (m, 2 H, CH₂Cl), 3.48 (m, 4 H,

2xCH₂N), 3.15 (s, 3 H, OSO₂CH₃); ¹³C NMR δ 167.11, 145.98, 146.34, 140.84, 136.05, 127.26, 122.22, 67.49, 54.35, 51.34, 41.36, 36.46. Anal. Calcd for C₁₂H₁₅ClN₄O₈S: C, 35.1; H, 3.7; N, 13.7; Cl, 8.5. Found: C, 35.7; H, 3.9; N, 13.6; Cl, 8.7%. Further elution gave 2-(2-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-

- 5 dinitroanilino)ethyl methanesulfonate (**13a**) (3.0 g, 80%): mp (EtOAc) 149-150 °C; ¹H NMR [(CD₃)₂SO] δ 8.73 (d, *J* = 2.8 Hz, 1 H, H-5), 8.35 (d, *J* = 2.9 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 8.00 (s, 1 H, CONH), 4.31 (m, 4 H, 2x CH₂OMs), 3.49 (m, 4 H, 2x CH₂-N), 3.14 (s, 6 H, 2xOSO₂CH₃). Anal. Calcd for C₁₃H₁₈N₄O₁₁S₂: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.7; H, 4.0; N, 11.8%.

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2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate (**IIb2**). A solution of dimesylate **13a** (1.62 g, 3.5 mmol) in warm EtOAc (100 mL) was treated with one portion of LiBr (400 mg, 4.7 mmol), and the mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was

15 adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:1 to 1:0) gave the dibromide (0.31 g, 20%) as yellow solid. (lit., foam) [Friedlos et al., *J. Med. Chem.* 1997, 1270]. Further elution gave **IIb2** (0.85 g, 53%): mp (EtOAc/petroleum ether) 153-154 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1 H, H-5), 8.33 (d, *J* = 2.8 Hz, 1 H, H-3), 8.19 (s, 1 H, CONH), 7.99 (s, 1 H, CONH),

20 4.29 (m, 2 H, CH₂OMs), 3.60 (m, 2 H, CH₂Br), 3.49 (m, 4 H, 2xCH₂-N), 3.14 (s, 3 H, OSO₂CH₃); ¹³C NMR δ 167.11, 145.75, 146.37, 140.92, 136.12, 127.24, 122.20, 67.53, 54.41, 51.16, 36.46, 29.73. Anal. Calcd for C₁₂H₁₅BrN₄O₈S: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.4; H, 3.4; N, 12.3; Br, 17.8%.

- 25 2-((2-Bromoethyl)-2-[(2-hydroxyethyl)amino]carbonyl)-4,6-dinitroanilino)ethyl methanesulfonate (**IIb3**). 2-Aminoethanol (2.9 g, 47 mmol) in 5 mL of water was added in one portion to a solution of crude 2-chloro-3,5-dinitrobenzoic acid chloride [prepared from 2-chloro-3,5-dinitrobenzoic acid **11** (5.0 g, 18.3 mmol) with SOCl₂] in Me₂CO (50 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then
- 30 acidified with 1N HCl to pH4-5 and concentrated under reduced pressure to remove the Me₂CO. EtOAc (100 mL) was added, and after 2h a white solid was collected, washed with EtOAc and air-dried to give 2-chloro-3,5-dinitro-*N*-(2-hydroxyethyl)benzamide (**12b**) (3.0 g, 36%): mp (EtOAc) 159-160 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.6

Hz, 1 H, H-5), 8.86 (m, 1 H, CONH), 8.56 (d, $J = 2.6$ Hz, 1 H, H-3), 4.83 (m, 1 H, -OH), 3.54 (m, 4 H) which was used for next step without further purification.

A solution of **12b** (0.6 g, 2.14 mmol) in CH_2Cl_2 was cooled in an ice-bath, and 3,4-dihydro-2H-pyran (2.0 mL) and p-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-N-[2-(tetrahydro-2H-pyran-2-yloxy)ethyl]benzamide (**12c**) (0.8 g, 100%): as an oil; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.67 (d, $J = 2.6$ Hz, 1 H, H-4), 8.60 (d, $J = 2.6$ Hz, 1 H, H-6), 7.02 (m, 1 H, CONH), 4.54 (m, 1 H), 4.00-3.50 (m, 6 H), 1.84-1.75 (m, 6 H) which was used for next step without further purification. Reaction of **12c** with diethanolamine, followed by MsCl/ Et_3N as described above, gave 2-[[2-[(methylsulfonyl)oxy]ethyl]-4,6-dinitro-6-([2-(tetrahydro-2H-pyran-2-yloxy)ethyl]amino)carbonyl]anilino]ethyl methanesulfonate (**13c**) (1.28 g, 100%): as a yellow foam; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.63 (d, $J = 2.9$ Hz, 1 H, H-5), 8.51 (d, $J = 2.9$ Hz, 1 H, H-3), 4.55 (m, 1 H), 4.39 (m, 4 H), 4.00-3.59 (m, 10 H), 3.15 (s, 3 H), 3.03 (s, 3 H), 1.64-1.39 (m, 6 H) which was used in the next step without further purification.

A solution of **13c** (1.28 g, 2.14 mmol) in THF (60 mL) was treated with 1 N HCl (40 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. NaHCO_3 , and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH (from 1:0 to 100:2), to give **13b** (0.84 g, 76%): as a yellow foam; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.78 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-5), 8.36 (d, $J = 2.7$ Hz, 1 H, H-3), 4.29 (m, 4 H, 2x CH_2OMs), 3.56 (m, 2 H), 3.45 (m, 6 H), 3.14 (s, 6 H, 2x OSO_2CH_3); ^{13}C NMR δ 165.37, 146.27, 145.06, 140.63, 135.78, 127.62, 122.32, 67.26, 59.17, 51.26, 42.14, 36.44.

Treatment of **13c** (0.49 g, 0.95 mmol) with LiBr (0.100 g, 1.2 mmol) in EtOAc (60 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave the dibromide (**14c**) (0.24 g, 53%). Further elution gave **11b3** (0.20 g, 42%): as yellow foam; ^1H NMR [$(\text{CD}_3)_2\text{SO}$] δ 8.77 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-5), 8.36 (d, $J = 2.7$ Hz, 1 H, H-3), 4.28

(m, 2 H, CH₂OMs), 3.58 (m, 4 H), 3.44 (m, 4 H), 3.14 (s, 3 H, OSO₂CH₃); ¹³C NMR δ 165.33, 145.79, 145.20, 140.87, 135.11, 127.50, 122.19, 67.49, 59.18, 54.21, 50.99, 42.09, 36.44, 29.68. HRMS *m/z* (M+1)⁺ required for C₁₄H₂₀⁷⁹BrN₄O₉S 499.01344; Found 499.01324.

5

- 2-((2-Iodoethyl)-2-[[2-(2-hydroxyethyl)amino]carbonyl]-4,6-dinitroanilino)ethyl methanesulfonate (**IIb4**). Treatment of **13b** (6.7 g, 13.0 mmol) with NaI (2.9 g, 20 mmol) in EtOAc (200 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether (from 2:1 to 1:0) gave 2-[bis(2-iodoethyl)amino]-*N*-(3-hydroxyethyl)-3,5-dinitrobenzamide (3.3 g, 44%) as a yellow solid: mp (EtOAc/petroleum ether) 129-131 °C; ¹H NMR [(CD₃)₂SO] δ 8.72 (d, *J* = 2.8 Hz, 1 H, H-4), 8.70 (m, 1 H, CONH), 8.32 (d, *J* = 2.8 Hz, 1 H, H-6), 4.80 (m, 1 H), 3.55 (m, 2 H), 3.43 (m, 4 H), 3.31 (m, 6 H); ¹³C NMR δ 165.3, 145.2, 144.7, 141.0, 136.3, 127.3, 122.0, 59.3, 54.7, 42.1, 2.94.
- 10 Later eluates gave **IIb4** (1.35 g, 19%) as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, *J* = 2.8 Hz, 1H, H-4), 8.74 (m, 1H, CONH), 8.34 (d, *J* = 2.8 Hz, 1 H, H-6), 4.28 (m, 2 H), 3.56 (m, 2 H), 3.43 (m, 2 H), 3.31 (m, 6 H), 3.13 (s, 3 H); ¹³C NMR δ 165.3, 145.5, 145.2, 140.8, 136.1, 127.4, 122.1, 67.5, 59.2, 55.4, 50.6, 42.1, 36.5, 2.6. HRMS (FAB) Calcd. For C₁₄H₂₀IN₄O₉S [M+H⁺] *m/z* 546.9996. Found; 546.9997.

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- 2-((2-Bromoethyl)-2-[[2-(2-hydroxypropyl)amino]carbonyl]-4,6-dinitroanilino)ethyl methanesulfonate (**IIb5**). A solution of **12d** (1.22 g, 4.0 mmol) in 50 mL of CH₂Cl₂ was cooled in an ice-bath, and 3,4-dihydro-2*H*-pyran (1.0 mL) and *p*-toluenesulfonic acid (0.1 g) were added. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. Chromatography of the residue on silica gel, eluting with EtOAc/petroleum ether (from 1:2 to 2:1), gave 2-chloro-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (**12e**) (1.45 g, 94%): as a pale yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.7 Hz, 1 H, H-4), 8.81 (m, 1 H, CONH), 8.51 (d, *J* = 2.7 Hz, 1 H, H-6), 4.57 (m, 1 H), 3.72 (m, 2 H), 3.46-3.25 (m, 4 H), 1.82-1.44 (m, 8 H).
- 25 ¹³C NMR δ 162.7, 148.4, 145.9, 140.3, 128.2, 125.8, 120.5, 98.0, 64.2, 61.3, 36.5, 30.2, 28.9, 24.9, 19.1. HRMS (FAB) Calcd. For C₁₅H₁₉³⁵ClN₃O₇ [M+H⁺] *m/z* 388.0912. Found; 388.0915.
- 30

- Reaction of **12e** (1.45 g, 3.75 mmol) with diethanolamine (1.67 g) as above gave 2-[bis(2-hydroxyethyl)amino]-3,5-dinitro-*N*-[2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]benzamide (1.62 g, 95%) as a yellow foam that was used directly; ¹H NMR [(CD₃)₂SO] δ 8.96 (m, 1H, CONH), 8.66 (d, J = 2.8 Hz, 1H, H-4), 8.31 (d, J = 2.8 Hz, 1H, H-6), 4.95 (m, 2H), 4.56 (m, 1H), 3.79-3.16 (m, 14H), 1.80-1.45 (m, 8 H); ¹³C NMR δ 166.2, 148.1, 143.6, 139.3, 133.8, 128.9, 123.8, 98.5, 64.8, 61.7, 58.5, 54.6, 37.3, 30.6, 29.2, 25.4, 19.6. HRMS (FAB) Calcd. For C₁₉H₂₉N₄O₆ [M+H]⁺ *m/z* 457.1935. Found; 457.1939.
- Reaction of the above diol (1.62 g, 3.55 mmol) with MsCl (2 mL) as above gave 2-[(methanesulfonyl)oxy]ethyl-4,6-dinitro-6-([2-(tetrahydro-2*H*-pyran-2-yloxy)propyl]-amino)carbonylanilinoethyl methanesulfonate (**13e**) (2.17 g, 100%): as a yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.71 (d, J = 2.8 Hz, 1H), 8.71 (m, 1H), 8.31 (d, J = 2.8 Hz, 1H), 4.26 (m, 4 H), 3.71-3.37 (m, 10 H), 3.13 (s, 6 H), 3.10 (m, 2 H), 1.82-1.43 (m, 8 H); ¹³C NMR δ 165.1, 146.3, 145.4, 140.9, 135.9, 127.4, 122.2, 98.0, 67.2, 64.3, 51.4, 45.7, 36.5, 30.2, 28.7, 24.9, 19.1, 8.5. HRMS (FAB) Calcd. For C₂₁H₃₃N₄O₁₃S₂ [M+H]⁺ *m/z* 613.1486. Found; 613.1481.
- A solution of **13e** (2.95 g, 3.55 mmol) in THF (120 mL) was treated with 1 N HCl (80 mL), and the solution was stirred at 20 °C for 1 h, then diluted with water (100 mL), neutralized with satd. NaHCO₃, and extracted with EtOAc (3x80 mL). The combined organic phases were washed with brine and dried, the solvent was evaporated, and the residue was purified by chromatography on silica gel, eluting with EtOAc/MeOH(100:1), to give 2-[(methanesulfonyl)oxy]ethyl-4,6-dinitro-6-([2-hydroxypropyl-amino)carbonylanilinoethyl methanesulfonate (**13d**) (1.4 g, 75%): as a yellow solid: mp (EtOAc/petroleum ether) 130-133 °C; ¹H NMR [(CD₃)₂SO] δ 8.74 (d, J = 2.8 Hz, 1H), 8.72 (m, 1H), 8.32 (d, J = 2.8 Hz, 1H), 4.29 (m, 4 H), 3.47 (m, 8 H), 3.14 (s, 6 H), 1.71 (m, 2 H); ¹³C NMR δ 165.2, 146.3, 145.3, 140.8, 135.9, 127.5, 122.3, 67.3, 58.4, 51.4, 36.8, 36.5, 31.7. Anal. (C₁₆H₂₄N₄O₁₂S₂) C, H, N.
- Treatment of **13d** (0.25 g, 0.45 mmol) with LiBr (53 mg, 0.7 mmol) in EtOAc (50 mL) at 60 °C for 3 h, and chromatography of the product on silica gel, eluting with

EtOAc/petroleum ether (from 2:1 to 1:0) gave **IIb5** (0.16 g, 66%): as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1H), 8.73 (m, 1H), 8.31 (d, $J = 2.8$ Hz, 1H), 4.28 (m, 2 H), 3.65-3.44 (m, 10 H), 3.13 (s, 3 H), 1.70 (m, 2 H); ^{13}C NMR δ 165.1, 145.7, 145.4, 141.0, 136.2, 127.3, 122.1, 67.5, 58.4, 51.1, 36.7, 36.5, 31.7, 29.6. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{22}^{79}\text{BrN}_4\text{O}_9\text{S}$ $[\text{M}+\text{H}^+]$ m/z 513.0291. Found; 513.0281.

2-((2-Bromoethyl)-2-[(2,3-dihydroxypropyl)amino]carbonyl)-4,6-dinitroanilino)ethyl methanesulfonate (IIb6). A solution of 2-(2-([(2,2-dimethyl-1,3-dioxolan-4-yl)methyl]amino)carbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13g**) [Palmer et al., *J. Med. Chem.* 1997, 40, 1272] (5.0 mmol) in MeOH (200 mL) was treated with p-toluenesulfonic acid (0.2 g) at room temperature for 4 h. Most of the MeOH was then evaporated, and the residue was taken up in EtOAc (200 mL), washed with satd. NaHCO_3 and brine, dried and concentrated. Chromatography of the product on silica gel, eluting with EtOAc/MeOH (20:1), gave 2-(2-[(2,3-dihydroxypropyl)amino]carbonyl){2-[(methylsulfonyl)oxy]ethyl}-4,6-dinitroanilino)ethyl methanesulfonate (**13f**) (2.0 g, 73%): as yellow foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.77 (m, 1 H, CONH), 8.74 (d, $J = 3.0$ Hz, 1 H, H-5), 8.37 (d, $J = 3.0$ Hz, 1 H, H-3), 4.30 (m, 4 H, $2\times\text{CH}_2\text{OMs}$), 3.66 (m, 1 H), 3.48-3.30 (m, 8 H), 3.14 (s, 6 H, $2\times\text{OSO}_2\text{CH}_3$); ^{13}C NMR δ 165.42, 146.24, 145.09, 140.60, 135.77, 127.67, 122.26, 69.77, 67.29, 63.87, 51.29, 42.98, 36.44. HRMS m/z ($\text{M}+1$) $^+$ required for $\text{C}_{16}\text{H}_{25}\text{N}_4\text{O}_{13}\text{S}_2$ 545.08596; Found 545.08680.

Treatment of **13f** (1.50 g, 2.75 mmol) with LiBr (0.21 g, 2.0 mmol) in EtOAc (60 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/MeOH 20:1), gave the dibromide **14f** (0.5 g, 35%) as a yellow foam and then **IIb6** (0.62 g, 34%): yellow solid; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (d, $J = 2.8$ Hz, 1 H, H-5), 8.71 (m, 1 H, CONH), 8.36 (d, $J = 2.8$ Hz, 1 H, H-3), 4.28 (m, 2 H, CH_2OMs), 3.69-3.30 (m, 11 H), 3.14 (s, 3 H); ^{13}C NMR δ 165.52, 145.87, 145.30, 140.93, 136.20, 127.64, 122.23, 68.89, 67.62, 63.93, 54.35, 51.08, 43.04, 36.52, 29.80. Anal. Calcd for $\text{C}_{15}\text{H}_{21}\text{BrN}_4\text{O}_{10}\text{S}$: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.0; H, 4.0; N, 10.5; Br, 15.2%. Further elution gave starting material **8e** (0.28, 19%).

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (**IIb7**). 2-Chloro-*N*-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (**12h**) (0.5 g, 1.34 mmol) was reacted with diethanolamine (0.5 g) in *p*-dioxane (10 mL) at room temperature for 3 h. The reaction mixture was poured into brine, extracted with EtOAc (3x70 mL), and the combined organic phases were dried and concentrated under reduced pressure to give crude 2-[bis(2-hydroxyethyl)amino]-*N*-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide. This was dissolved in CH₂Cl₂ (100 mL), cooled in an ice-bath, and treated with Et₃N (1.5 mL) followed by MsCl (0.7 mL) in one portion. After stirring for 10 min, sat. NaHCO₃ (100 mL) was added and the mixture was stirred for a further 30 min, then the aqueous phase was extracted with CH₂Cl₂ (2x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was purified by column chromatography, eluting with EtOAc/MeOH (20:1 to 9:0) to give yield 2-[(methanesulfonyl)oxyethyl]-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate (**13h**) (0.75 g, 93%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-5), 8.20 (d, *J* = 2.7 Hz, 1 H, H-3), 4.28 (m, 4 H, 2x CH₂OMs), 3.56 (m, 5 H), 3.44 (m, 5 H), 3.15 (s, 6 H), 2.35 (m, 6 H), 1.71 (m, 2 H).

A solution of **13h** (0.70 g, 1.17 mmol) in EtOAc (100 mL) was treated with LiBr (118 mg, 1.36 mmol) at 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/MeOH (from 20:1 to 10:1) gave 2-[bis(2-bromoethyl)amino]-*N*-[3-(4-morpholinyl)propyl]-3,5-dinitrobenzamide (**14h**) 228 mg (34%) as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.76 (d, *J* = 2.8 Hz, 1 H, H-5), 8.30 (d, *J* = 2.8 Hz, 1 H, H-3), 3.58-3.42 (m, 14 H), 2.36 (m, 6 H), 1.70 (m, 2 H); ¹³C NMR δ 165.08, 145.57, 145.27, 141.19, 136.40, 127.27, 122.10, 66.08, 59.66, 55.64, 53.19, 37.61, 25.39, 13.99. HRMS *m/z* (*M*+1)⁺ required for C₁₈H₂₅⁷⁹Br₂N₅O₆: 566.0250. Found; 566.0241. Later eluates gave **IIb7** (300 mg, 44%); as yellow foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (m, 1 H, CONH), 8.75 (d, *J* = 2.6 Hz, 1 H, H-4), 8.31 (d, *J* = 2.6 Hz, 1 H, H-6), 4.28 (m, 2 H, CH₂OMs), 3.56 (m, 7 H), 3.44 (m, 5 H), 3.14 (s, 3 H), 2.35 (m, 6 H), 1.71 (m, 2 H); ¹³C NMR δ 165.07, 145.79, 145.31, 140.92, 136.04, 127.36, 122.21, 67.50, 66.09, 59.64, 55.68, 53.21, 51.10, 37.63, 36.45, 25.41, 14.00. HRMS *m/z*

(M+1)⁺ required for C₁₉H₂₉⁷⁹BrN₅O₉S 582.08519. Found 582.08694; together with starting material **13h** (117 mg, 18%).

Methyl 3-{[2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate (IIb8). Methyl alanine hydrochloride (2.55 g, 18.3 mmol) was dissolved in water (12 mL), and the solution was diluted with Me₂CO (20 mL) and Et₂O (50 mL). This was then poured into a solution of crude 2-chloro-3,5-dinitrobenzoyl chloride [prepared from 2-chloro-3,5-dinitrobenzoic acid **11** (5.0 g, 18.3 mmol) with SOCl₂] in Me₂CO (50 mL) while cooling in an ice-bath. The mixture was stirred for 30 min, then poured into water and extracted with EtOAc. The organic phase was washed with satd. NaHCO₃ and brine, dried, and concentrated to give methyl 3-[(2-chloro-3,5-dinitrobenzoyl)amino]propanoate (**12i**) (4.45 g, 73.3%): mp (EtOAc/petroleum ether) 128-130 °C; ¹H NMR [(CD₃)₂SO] δ 8.99 (d, *J* = 2.7 Hz, 1 H, H-4), 8.96 (m, 1 H, CONH), 8.51 (d, *J* = 2.7 Hz, 1 H, H-6), 3.63 (s, 3 H, CO₂CH₃), 3.50 (m, 2 H, CONHCH₂), 2.64 (m, 2 H, CH₂CO₂). The product was used without further purification.

A mixture of **12i** (2.5 g, 7.6 mmol) and diethanolamine (2.0 g) in *p*-dioxane (30 mL) was kept at room temperature for 3 h, then poured into brine and extracted with EtOAc (3x70 mL). The combined organic phases were dried and evaporated under reduced pressure. The residue was dissolved in CH₂Cl₂ (15 mL), cooled in an ice-bath, and treated with Et₃N (8 mL) and MsCl (4 mL). After stirring for 10 min, satd. NaHCO₃ (100 mL) was added, and following a further 30 min of stirring the aqueous phase was extracted with CH₂Cl₂ (2x70 mL). The combined organic phases were dried and then evaporated under reduced pressure, and the residue was then purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0) gave **IIb8** (0.2 g, 5%): as yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.88 (m, 1 H, CONH), 8.74 (d, *J* = 2.7 Hz, 1 H, H-4), 8.31 (d, *J* = 2.7 Hz, 1 H, H-6), 4.29 (m, 2 H, CH₂OMs), 3.71 (m, 2 H, CH₂Cl), 3.63 (s, 3 H, CO₂CH₃), 3.54 – 3.36 (m, 6 H), 3.14 (s, 3 H, OSO₂CH₃), 2.65 (m, 2 H, CH₂CO₂); ¹³C NMR δ 171.68, 165.34, 146.14, 145.17, 140.74, 135.59, 127.58, 122.42, 67.47, 54.22, 51.45, 51.22, 41.37, 36.48, 35.44, 32.95. HRMS *m/z* (M+1)⁺ required for C₁₆H₂₂³⁵ClN₄O₁₀S; 497.0745. Found; 497.0748.

Further elution gave methyl 3-{[2-bis{2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate (**13i**) (2.6 g, 62%): as yellow oil; ¹H NMR

$[(CD_3)_2SO]$ δ 8.90 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.32 (d, $J = 2.7$ Hz, 1 H, H-6), 4.30 (m, 4 H, $2 \times CH_2OMs$), 3.63 (s, 3 H, CO_2CH_3), 3.52 (m, 2 H, $CONHCH_2$), 3.44 (m, 4 H, $2 \times CH_2N$), 3.14 (s, 6 H, $2 \times OSO_2CH_3$), 2.65 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.66, 165.28, 146.36, 144.98, 140.52, 135.23, 127.64, 122.50, 67.20, 51.40, 51.25, 36.44, 35.45, 32.91. HRMS m/z $(M+1)^+$ required for $C_{17}H_{25}N_4O_{13}S_2$: 557.0860. Found: 557.0853.

In an alternative preparation of **IIb8**, a solution of **13i** (0.417 g, 0.75 mmol) in DMF (10 mL) was treated with LiCl (0.038 g, 1.00 mmol) at 60 °C for 2 h, and then cooled and poured into dilute HCl and extracted with EtOAc (3x80 mL). Workup and chromatography of the product on silica gel, eluting with EtOAc/petroleum ether from 1:1 to 2:1, gave methyl 3-({2-[bis(2-chloroethyl)amino]-3,5-dinitrobenzoyl}amino)propanoate (**15i**) (0.16 g, 51%): as yellow oil; 1H NMR $[(CD_3)_2SO]$ δ 8.85 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.68 (m, 4 H, $2 \times CH_2Cl$), 3.63 (s, 3 H, CO_2CH_3), 3.50 (m, 2 H, $CONHCH_2$), 3.41 (m, 4 H, $N(CH_2)_2$), 2.64 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.59, 165.28, 145.81, 145.31, 140.89, 135.89, 127.45, 122.26, 54.08, 51.40, 41.51, 35.35, 32.92. Further elution then gave **IIb8** (0.124 g, 33%), identical with the sample prepared above.

Methyl 3-{{2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl}amino}propanoate (IIb9**)**. Treatment of **13i** (2.04 g, 3.67 mmol) with LiBr (0.318 g, 3.67 mmol) in EtOAc (100 mL) at 60 °C for 3 h, followed by chromatography on silica gel and elution with EtOAc/petroleum ether from 1:1 to 1:0) gave methyl 3-({2-[bis(2-bromoethyl)amino]-3,5-dinitrobenzoyl}amino)propanoate (**14i**) (0.55 g, 29%): as yellow foam; 1H NMR $[(CD_3)_2SO]$ δ 8.86 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.29 (d, $J = 2.7$ Hz, 1 H, H-6), 3.63 (s, 3 H, CO_2CH_3), 3.60 – 3.43 (m, 10 H), 2.64 (m, 2 H, CH_2CO_2); ^{13}C NMR δ 171.60, 165.28, 145.39, 145.36, 141.07, 136.05, 127.44, 122.25, 53.97, 51.44, 35.35, 32.95, 29.96. HRMS m/z $(M+1)^+$ required for $C_{15}H_{19}^{79}Br_2N_4O_7$: 524.9621. Found: 524.9616.

Further elution gave **IIb9** (0.96 g, 48%): as yellow foam; 1H NMR $[(CD_3)_2SO]$ δ 8.89 (m, 1 H, CONH), 8.74 (d, $J = 2.7$ Hz, 1 H, H-4), 8.31 (d, $J = 2.7$ Hz, 1 H, H-6), 4.28 (m, 2 H, CH_2OMs), 3.63 (s, 3 H, CO_2CH_3), 3.60 – 3.43 (m, 8 H), 3.14 (s, 3 H,

OSO₂CH₃), 2.65 (m, 2 H, CH₂CO₂); ¹³C NMR δ 171.63, 165.28, 145.87, 145.19, 140.81, 135.65, 127.54, 122.37, 67.47, 54.25, 51.42, 51.02, 36.45, 35.40, 32.93, 29.69. HRMS *m/z* (M+1)⁺ required for C₁₆H₂₂⁷⁹BrN₄O₁₀S: 541.0240. Found; 541.0228, followed by starting material **13g** (0.45 g, 22%).

5

Example C : Preparation of analogues of class IIc by the method outlined in Scheme 3.

10 **2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc1).**

A solution of methyl 3-[bis(2-hydroxyethyl)amino]-2,6-dinitrobenzoate [Palmer et al., *J. Med. Chem.* 1996, 39, 2518] (7.24 g, 22 mmol) in CH₂Cl₂ (120 mL) was cooled in an ice-bath and Et₃N (15 mL) and MsCl (8 mL) were added in one portion. After stirred for 10 min, satd. NaHCO₃ (100 mL) was added, and after a further 30 min the aqueous
15 phase was extracted with CH₂Cl₂ (2x70 mL), the combined organic phase were dried, concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel. Elution with EtOAc/petroleum ether (1:1 to 1:0), gave crude methyl 3-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,6-dinitrobenzoate (**16**)
(10.67 g, 100%) as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.32 (d, *J* = 9.6 Hz, 1 H, H-5),
20 7.75 (d, *J* = 9.6 Hz, 1 H, H-4), 4.32 (m, 4 H), 3.88 (s, 3 H), 3.67 (m, 4 H), 3.14 (m, 6 H); ¹³C NMR δ 163.02, 147.59, 138.40, 136.46, 128.33, 125.83, 123.96, 66.73, 54.00, 50.24, 45.58, 36.58.

Hydrolysis of **16** (10.6 g, 21.9 mmol) with 3 N KOH (40 mL) in dioxane (200 mL) at
25 room temperature for 15 min, followed by acidification with 1 N HCl and extraction with EtOAc, gave a quantitative yield of crude 3-(bis{2-[(methylsulfonyl)oxy]ethyl}amino)-2,6-dinitrobenzoic acid (**17**): mp 200-210 °C, HRMS: C₁₃H₁₈N₃O₁₂ S₂ requires *m/z* 472.0332. Found: 472.033, that was used without purification. The acid chloride (SOCl₂/cat. DMF) from **17** (3.2 g, 6.8 mmol) was
30 dissolved in Me₂CO (30 mL), cooled in an ice-bath and treated with concentrated NH₄OH (10 mL). After stirring for 10 min. the reaction mixture was acidified to pH 2-3 with 1 N HCl, then most of the solvent was evaporated and the residue was partitioned between EtOAc and water. The aqueous layer was extracted with EtOAc

(2x80 mL) and the combined organic extracts were dried and evaporated under reduced pressure. The residue was adsorbed directly onto silica gel and chromatographed.

Elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave **IIc1** (0.145 g, 5.2%: mp (EtOAc) 134-136 °C; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, *J* = 9.3 Hz, 1 H, H-6), 4.27 (m, 2 H, CH₂OMs), 3.73 (m, 2 H), 3.66 (m, 2 H), 3.59 (m, 2 H), 3.15 (s, 3 H); ¹³C NMR δ 163.06, 146.40, 140.52, 137.65, 129.42, 127.51, 122.89, 66.83, 52.93, 50.16, 41.45, 36.57. Anal. Calcd. For C₁₂H₁₅ClN₄O₈S: C, 35.1; H, 3.7; N, 13.6; Cl, 8.6. Found: C, 35.5; H, 3.7; N, 13.6; Cl, 8.6%.

Elution of the column with EtOAc/MeOH (50:1) gave 2-(3-(aminocarbonyl){2-[(methylsulfonyl)oxy]ethyl}-2,6-dinitroanilino)ethyl methanesulfonate (**18a**) (1.1 g, 34%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ¹H NMR [(CD₃)₂SO] δ 8.26 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.66 (d, *J* = 9.3 Hz, 1 H, H-6), 4.27 (m, 4 H, 2x-CH₂OMs), 3.63 (m, 4 H, 2x-CH₂N), 3.15 (s, 6 H, 2x CH₃SO₃-); ¹³C NMR δ 163.00, 146.51, 140.98, 137.99, 129.30, 127.47, 123.40, 66.74, 50.44, 36.56. Anal. Calcd. For C₁₃H₁₈N₄O₁₁S₂: C, 33.2; H, 3.9; N, 11.9. Found: C, 33.5; H, 3.8; N, 11.9%.

2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate

(IIc2). LiBr (117 mg, 1.34 mmol) was added in one portion to a solution of **18a** (0.474 g, 1.0 mmol) in Me₂CO/EtOAc (1:1, 100 mL), and the reaction mixture was heated to 60 °C for 2 h. Volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and chromatographed. Elution with EtOAc/petroleum ether (1:1) gave 3-[bis(2-bromoethyl)amino]-2,6-dinitrobenzamide (**19a**) (95 mg, 21%): as a yellow oil; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.5 Hz, 1 H, H-5), 8.22 (s, 1 H, NH), 7.88 (s, 1 H, NH), 7.63 (d, *J* = 9.5 Hz, 1 H, H-4), 3.68 (m, 4 H), 3.58 (m, 4 H (Lit. [Palmer et al., J. Med. Chem., 1996, 39, 2518-2528]).

Further elution with EtOAc/petroleum ether (3:1) gave **IIc2** (208 mg, 46%): mp (EtOAc/petroleum ether) 143-145 °C; ¹H NMR [(CD₃)₂SO] δ 8.25 (d, *J* = 9.3 Hz, 1 H, H-5), 8.23 (s, 1 H, NH), 7.89 (s, 1 H, NH), 7.64 (d, *J* = 9.3 Hz, 1 H, H-6), 4.28 (m, 2 H, CH₂OMs), 3.67 (m, 4 H), 3.57 (m, 2 H), 3.16 (s, 3 H); ¹³C NMR δ 163.05, 146.17, 140.49, 137.68, 129.42, 127.53, 122.89, 66.85, 52.92, 50.04, 36.57, 29.95. Anal. Calcd.

For $C_{12}H_{15}BrN_4O_8S$: C, 31.7; H, 3.3; N, 12.3; Br, 17.4. Found: C, 31.9; H, 3.3; N, 12.2; Br, 17.5%.

Later eluates gave starting material **18a** (150 mg).

- 5 **2-((2-Bromoethyl)-3-[[2-hydroxyethyl]amino]carbonyl)-2,6-dinitroanilino)ethyl methanesulfonate (IIc3)**. Treatment of 3-(3-[[2-hydroxyethyl]amino]carbonyl}{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino-1-methylpropyl methanesulfonate (**18b**) (310 mg, 0.6 mmol) in EtOAc (50 mL) with LiBr (78 mg, 0.9 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to
10 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(2-hydroxyethyl)-2,6-dinitrobenzamide (**19b**) (70 mg, 25%) as a foam; 1H NMR $[(CD_3)_2SO]$ δ 8.80 (m, 1H, CONH), 8.24 (d, $J=9.4$ Hz, 1H), 7.63 (d, $J=9.4$ Hz, 1H), 4.66 (m, 1H), 3.70 (m, 4H), 3.60 (m, 4H), 3.45 (m, 2H), 3.22 (m, 2H); ^{13}C NMR δ 161.4, 145.8, 140.2, 137.5, 129.2, 127.6, 122.6, 59.0, 52.6, 41.7, 30.0. HRMS (FAB) Calcd. For $C_{13}H_{17}^{79}Br_2N_4O_6$ $[M+H]^+$ m/z 482.9515.
15 Found; 482.9508.

- Further elution with EtOAc/MeOH (50:2) gave **IIc3** (118 mg, 39%): mp. 94-97 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.80 (m, 1H, CONH), 8.25 (d, $J=9.4$ Hz, 1H), 7.64 (d, $J=9.4$ Hz, 1H), 4.67 (m, 1H), 4.27 (m, 2H), 3.63 (m, 4H), 3.57 (m, 2H), 3.45 (m, 2H), 3.26 (m,
20 2H), 3.15 (s, 3H); ^{13}C NMR δ 161.4, 146.2, 140.5, 137.7, 129.2, 127.5, 122.9, 66.8, 59.0, 50.0, 41.7, 36.6, 29.9. Anal. ($C_{14}H_{19}BrN_4O_9S$) C, H, N.

- 2-((2-Chloroethyl)-3-[[3-hydroxypropyl]amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIc4)**. Reaction of the acid chloride of **17** with 3-aminopropanol in
25 Me_2CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/petroleum ether (1:1), gave **IIc4** (292 mg, 12%): mp (EtOAc/petroleum ether) 104-109 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.75 (t, $J=5.8$ Hz, 1H, CONH), 8.24 (d, $J=9.4$ Hz, 1H, H-5), 7.64 (d, $J=9.4$ Hz, 1H, H-6), 4.44 (m, 1H, CHO H), 4.26 (m, 2H), 3.72 (m, 2H), 3.65 (m, 2H), 3.59 (m, 2H), 3.43 (m, 2H), 3.20 (m,
30 2H), 3.15 (s, 3H), 1.60 (m, 2H); ^{13}C NMR δ 161.09, 146.42, 140.49, 137.65, 129.23, 127.58, 122.91, 66.82, 58.22, 52.88, 50.11, 41.44, 36.57, 36.37, 31.57. Anal. Calcd. For $C_{15}H_{21}ClN_4O_9S$: C, 38.5; H, 4.5; N, 12.0; Cl, 7.5. Found: C, 38.8; H, 4.8; N, 11.5; Cl, 7.0%.

Further elution with EtOAc gave 2-(3-[[3-(hydroxypropyl)amino]carbonyl]{2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**18e**) (1.1 g, 41%): mp (EtOAc/MeOH/petroleum ether) 160-162 °C; ¹H NMR [(CD₃)₂SO] δ 8.77 (t, *J* = 5.8 Hz, 1 H, CONH), 8.26 (d, *J* = 9.4 Hz, 1 H, H-5), 7.66 (d, *J* = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 4 H, 2xCH₂OMs), 3.63 (m, 4 H, 2xCH₂N), 3.43 (m, 2 H), 3.20 (m, 2 H), 3.15 (s, 6 H, 2xCH₃SO₃), 1.60 (m, 2 H); ¹³C NMR δ 161.03, 146.52, 140.95, 138.00, 129.12, 127.54, 123.42, 66.72, 58.22, 50.39, 36.55, 36.37, 31.57. Anal. Calcd. For C₁₆H₂₄N₄O₁₂S₂: C, 36.4; H, 4.6; N, 10.6. Found: C, 36.6; H, 4.5; N, 10.6%.

2-((2-Bromoethyl)-3-[[3-(hydroxypropyl)amino]carbonyl]-2,6-dinitroanilino)ethyl methanesulfonate (**IIc5**). Treatment of **18c** (716 mg, 1.36 mmol) in EtOAc (200 mL) with LiBr ((175 mg, 2.0 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/ petroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(3-(hydroxypropyl))-2,6-dinitrobenzamide (**19c**) (289 mg, 42%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.8 Hz, 1 H, CONH), 8.23 (d, *J* = 9.4 Hz, 1 H, H-5), 7.62 (d, *J* = 9.4 Hz, 1 H, H-4), 4.47 (m, 1 H, CHOH), 3.68 (m, 4 H), 3.57 (m, 4 H), 3.43 (m, 2 H), 3.20 (m, 2 H), 1.60 (m, 2 H); ¹³C NMR δ 161.20, 146.90, 140.20, 137.53, 129.36, 127.69, 122.56, 58.29, 52.64, 36.42, 31.61, 30.13. HRMS *m/z* (*M*+1)⁺ required for C₁₄H₁₉⁷⁹Br₂N₄O₆: 496.9671. Found: 496.9667.

Further elution with EtOAc/MeOH (50:2) gave **IIc5** (270 mg, 39%): mp. 115-117 °C; ¹H NMR [(CD₃)₂SO] δ 8.75 (t, *J* = 5.8 Hz, 1 H, CONH), 8.24 (d, *J* = 9.4 Hz, 1 H, H-5), 7.64 (d, *J* = 9.4 Hz, 1 H, H-6), 4.43 (m, 1 H, CHOH), 4.27 (m, 2 H, CH₂OMs), 3.66 (m, 4 H, 2xCH₂N), 3.59 (m, 2 H), 3.44 (m, 2 H), 3.22 (m, 2 H), 3.15 (s, 3 H, CH₃SO₃), 1.60 (m, 2 H); ¹³C NMR δ 161.08, 146.19, 140.47, 137.69, 129.24, 127.59, 122.91, 66.83, 58.22, 52.87, 50.00, 36.57, 36.37, 31.58, 29.95. Anal. Calcd. For C₁₅H₂₁BrN₄O₉S: C, 35.2; H, 4.1; N, 10.9; Br, 15.4. Found: C, 35.4; H, 3.9; N, 11.0; Br, 16.3%.

2-((2-Bromoethyl)-3-[[4-(hydroxybutyl)amino]carbonyl]-2,6-dinitroanilino)ethyl methanesulfonate (**IIc6**). Treatment of 3-(3-[[4-(hydroxybutyl)amino]carbonyl]{3-[(methylsulfonyl)oxy]butyl}-2,4-dinitroanilino)-1-methylpropyl methanesulfonate (**18d**) (500 mg, 0.92 mmol) in EtOAc (100 mL) with LiBr (110 mg, 1.4 mmol), followed by chromatography on silica gel and elution with EtOAc/petroleum ether (from 1:1 to 1:0) gave 3-[bis(2-bromoethyl)amino]-*N*-(4-(hydroxybutyl))-2,6-dinitrobenzamide (**19d**) (100

mg, 21%) as a foam; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.73 (m, 1H, CONH), 8.25 (d, $J=9.4$ Hz, 1H), 7.63 (d, $J=9.4$ Hz, 1H), 4.38 (m, 1H), 3.69 (m, 4H), 3.57 (m, 4H), 3.40 (m, 2H), 3.14 (m, 2H), 1.47 (m, 4H); ^{13}C NMR δ 161.0, 145.8, 140.2, 137.6, 129.3, 127.6, 122.6, 60.2, 52.6, 30.0, 29.6, 24.8. HRMS (FAB) Calcd. For $\text{C}_{15}\text{H}_{20}^{79}\text{Br}_2\text{N}_4\text{O}_6$ $[\text{M}+\text{H}^+]$
 5 m/z 510.9828. Found; 510.9819.

Further elution with EtOAc/MeOH (50:2) gave **IIc6** (117 mg, 30%): mp. 114-117 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.74 (m, 1H, CONH), 8.25 (d, $J=9.4$ Hz, 1H), 7.65 (d, $J=9.4$ Hz, 1H), 4.37 (m, 1H), 4.27 (m, 2H), 3.65 (m, 4H), 3.57 (m, 2H), 3.35 (m, 2H), 3.16
 10 (m, 2H), 3.15 (s, 3H), 1.47 (m, 4H); ^{13}C NMR δ 160.0, 146.1, 140.6, 137.8, 129.2, 127.5, 122.9, 66.8, 60.2, 52.9, 50.0, 36.6, 29.9, 29.6, 24.9. Anal. ($\text{C}_{16}\text{H}_{23}\text{BrN}_4\text{O}_9\text{S}$) C, H, N.

2-((2-Chloroethyl)-3-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-
 15 **dinitroanilino)ethyl methanesulfonate (IIc7).** Reaction of the acid chloride of 17 (2.4 g, 5.1 mmol) with 3-amino-1,2-propanediol Me_2CO at 0 °C as described above, followed by chromatography of the product on silica gel and elution with EtOAc, gave **IIc7** (240 mg, 10%): mp (EtOAc) 100-105 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.77 (t, $J=5.8$ Hz, 1H, CONH), 8.24 (d, $J=9.4$ Hz, 1H, H-5), 7.64 (d, $J=9.4$ Hz, 1H, H-6), 4.72 (d,
 20 $J=4.9$, 1H, CHOH), 4.52 (t, $J=5.7$, 1H, CH_2OH), 4.27 (m, 2H, CH_2OMs), 3.74 – 3.50 (m, 10H), 3.15 (s, 3H, CH_3SO_3), 3.04 (m, 1H); ^{13}C NMR δ 161.48, 146.38, 140.55, 137.73, 129.28, 127.51, 122.88, 69.89, 66.83, 63.57, 52.95, 50.17, 42.55, 41.43, 36.58. Anal. Calcd. For $\text{C}_{15}\text{H}_{21}\text{ClN}_4\text{O}_{10}\text{S}$: C, 37.2; H, 4.4; N, 11.6; Cl, 7.2. Found: C, 38.0; H, 4.5; N, 11.1; Cl, 7.2%.

25 Further elution with EtOAc/MeOH (50 : 1) gave 2-(3-[(2,3-dihydroxypropyl)amino]carbonyl) {2-[(methylsulfonyl)oxy]ethyl}-2,4-dinitroanilino)ethyl methanesulfonate (**18e**) (480 mg, 51%): mp (MeOH/EtOAc) 60-63 °C; ^1H NMR $[(\text{CD}_3)_2\text{SO}]$ δ 8.78 (t, $J=5.8$ Hz, 1H, CONH), 8.24 (d, $J=9.4$ Hz, 1H, H-5), 7.66 (d, $J=9.4$ Hz, 1H, H-6), 4.72 (d, $J=4.9$, 1H, CHOH), 4.52 (t, $J=5.7$, 1H, CH_2OH), 4.27 (m, 4H, $2\times\text{CH}_2\text{OMs}$), 3.63 (m, 4H), 3.52 – 3.30 (m, 5H), 3.15 (s, 3H, $2\times\text{CH}_3\text{SO}_3$), 3.06 (m, 1H); ^{13}C NMR δ 161.43, 146.49, 141.01, 138.07, 129.15, 127.47,

123.36, 69.89, 66.73, 63.67, 50.44, 42.55, 36.56. Anal. Calcd. For $C_{16}H_{24}N_4O_{13}S_2$: C, 35.3; H, 4.5; N, 10.3. Found: C, 35.8; H, 4.5; N, 10.5%.

- 2-((2-Bromoethyl)-3-[(2,3-dihydroxypropyl)amino]carbonyl)-2,4-dinitroanilino)ethyl methanesulfonate (IIc8).** Treatment of **18e** (0.92 g, 1.7 mmol) in EtOAc (200 mL) with LiBr (170 mg, 1.95 mmol) as above, followed by chromatography on silica gel and elution with EtOAc/MeOH (50:1), gave 3-[bis(2-bromoethyl)amino]-*N*-(2,3-dihydroxypropyl)-2,4-dinitrobenzamide (**19e**) (155 mg, 18%) as yellow oil; 1H NMR $[(CD_3)_2SO]$ δ 8.76 (t, $J = 5.8$ Hz, 1 H, CONH), 8.23 (d, $J = 9.5$ Hz, 1 H, H-5), 7.63 (d, $J = 9.5$ Hz, 1 H, H-6), 4.72 (d, $J = 5.1$ Hz, 1 H, CHOH), 4.52 (t, $J = 5.7$ Hz, 1 H, CH_2OH), 3.70 – 3.50 (m, 11 H), 3.04 (m, 1 H). HRMS m/z (M+1)⁺ required for $C_{14}H_{19}^{79}Br_2N_4O_7$: 512.9621. Found; 512.9603.
- Further elution gave **IIc8** (278 mg, 31%): mp (EtOAc) 108–110 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.77 (t, $J = 5.8$ Hz, 1 H, CONH), 8.24 (d, $J = 9.4$ Hz, 1 H, H-5), 7.64 (d, $J = 9.4$ Hz, 1 H, H-6), 4.72 (d, $J = 4.9$, 1 H, CHOH), 4.52 (t, $J = 5.7$, 1 H, CH_2OH), 4.27 (m, 2 H, CH_2OMs), 3.70 – 3.50 (m, 10 H), 3.15 (s, 3 H, CH_3SO_3), 3.06 (m, 1 H); ^{13}C NMR δ 161.47, 146.16, 140.52, 137.77, 129.28, 127.53, 122.88, 69.89, 66.84, 63.57, 52.94, 50.05, 42.55, 36.58, 29.94. Anal. Calcd. For $C_{15}H_{21}BrN_4O_{10}S$: C, 34.1; H, 4.0; N, 10.6; Br, 15.0. Found: C, 34.3; H, 4.1; N, 10.4; Br, 15.4%.
- And starting material (200 mg, 22%)

- 2-[(2-Chloroethyl)-3-([3-(4-morpholinyl)propyl]amino)carbonyl]-2,4-dinitroanilino)ethyl methanesulfonate (IIc9).** Reaction of the acid chloride from **17** (1.3 g) in Me_2CO with 3-(4-morpholinyl)propylamine (1.0 mL) at 0°C as described above, followed by chromatography of the product on silica gel and elution with EtOAc/MeOH (9:1 to 4:1), gave **IIc9** (0.37 g, 25%): mp (EtOAc/petroleum ether) 113–116 °C; 1H NMR $[(CD_3)_2SO]$ δ 8.79 (t, $J = 5.6$ Hz, 1 H, CONH), 8.25 (d, $J = 9.4$ Hz, 1 H, H-5), 7.65 (d, $J = 9.4$ Hz, 1 H, H-6), 4.28 (t, $J = 5.3$, 2 H), 3.73 (t, $J = 6.3$, 2 H), 3.66 (t, $J = 5.2$, 2 H), 3.60 (t, $J = 5.9$, 2 H), 3.56 (m, 4H), 3.17 (m, 5 H), 2.34 (m, 6H), 1.61 (m, 2H); ^{13}C NMR δ 161.07, 146.44, 140.44, 137.62, 129.23, 127.60, 122.92, 66.81, 66.12, 55.40, 53.19, 52.85, 50.10, 41.45, 37.30, 36.56, 25.12. HRMS m/z (M+1)⁺ requires $C_{19}H_{29}^{35}ClN_5O_9S$: 538.13745. Found: 538.13869.

Later eluates gave 2-[{2-[(methylsulfonyl)oxy]ethyl}-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (**18f**) (0.93 g, 56%) as a yellow solid, mp (EtOAc/petroleum ether) 90-95 °C; ¹H NMR [(CD₃)₂SO] δ 8.79 (t, *J* = 5.7 Hz, 1 H, CONH), 8.25 (d, *J* = 9.4 Hz, 1 H, H-5), 7.65 (d, *J* = 9.4 Hz, 1 H, H-6), 4.28 (t, *J* = 5.3, 4 H), 3.64 (t, *J* = 5.2, 4 H), 3.55 (t, *J* = 4.6, 4 H), 3.15 (m, 8 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ¹³C NMR δ 161.03, 146.55, 140.90, 137.97, 129.10, 127.56, 123.43, 66.72, 66.12, 55.39, 53.19, 50.37, 37.29, 36.55, 25.13. HRMS *m/z* (M+1)⁺ requires C₂₀H₃₂N₅O₁₂S₂: 598.14889. Found: 598.14894.

- 10 **2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate (IIc10)**. LiBr (107 mg, 1.3 mmol) was added in one portion to a warm solution of **18f** (0.53 g, 0.89 mmol) in EtOAc (50 mL). The reaction mixture was heated to 60 °C for 2 h, then volatiles were removed under reduced pressure, and the residue was adsorbed directly onto silica gel and
- 15 chromatographed. Elution with EtOAc/MeOH (10:1 to 5:1) gave 3-[bis(2-bromoethyl)amino]-*N*-[3-(4-morpholinyl)propyl]-2,6-dinitrobenzamide (**19f**) (109 mg, 22%) as a foam; ¹H NMR [(CD₃)₂SO] δ 8.77 (t, *J* = 5.6 Hz, 1 H, CONH), 8.23 (d, *J* = 9.4 Hz, 1 H, H-5), 7.63 (d, *J* = 9.4 Hz, 1 H, H-6), 3.68 (m, 4H), 3.57 (m, 8 H), 3.17 (m, 2 H), 2.34 (m, 6 H), 1.61 (m, 2 H). HRMS: C₁₅H₁₁⁷⁹Br₂N₄O₅ requires *m/z* 438.9253.
- 20 Found: 438.9228.

Later eluates gave **IIc10** (293 mg, 57%): mp (EtOAc/petroleum ether) 114-117 °C; ¹H NMR [(CD₃)₂SO] δ 8.79 (t, *J* = 5.6 Hz, 1 H, CONH), 8.25 (d, *J* = 9.4 Hz, 1 H, H-5), 7.65 (d, *J* = 9.4 Hz, 1 H, H-6), 4.28 (t, *J* = 5.2, 2 H), 3.66 (m, *J* = 5.2, 4H), 3.56 (m, *J* = 4.6, 6 H), 3.17 (m, 5 H), 2.34 (m, 6 H), 1.61 (m, 2 H); ¹³C NMR δ 161.07, 146.22, 140.39, 137.65, 129.21, 127.62, 122.92, 66.83, 66.07, 55.37, 53.15, 52.83, 49.99, 37.28, 36.57, 29.97, 25.07. HRMS *m/z* (M+1)⁺ requires C₁₉H₂₉⁷⁹BrN₅O₉S: 582.08694. Found: 582.08639.

Later eluates gave starting material **18f** (124 mg, 23%).

- 30 The following Tables 2 and 3 give biological data for the compounds listed in Table 1.

Table 2. Relative cytotoxicities of selected examples of the compounds of Table 1 in NTR-transfected cell lines (18 h exposure).

| No | Human ovarian ^a | | | Human colon ^b | | |
|--------------------------------|-------------------------------|-------|--------------------|-------------------------------|-------------------|--------------------|
| | IC ₅₀ ^d | | Ratio ^e | IC ₅₀ ^d | | Ratio ^e |
| | NR- | NR+ | | NR- | NR+ | |
| <i>Examples of formula IIa</i> | | | | | | |
| IIa2 | 226 | 0.84 | 280 | 150 | 0.69 | 235 |
| IIa3 | 135 | 0.19 | 715 | 80 | 0.22 | 387 |
| IIa4 | 97 | 0.33 | 311 | 70 | 0.41 | 172 |
| IIa5 | 286 | 0.61 | 470 | 192 | 0.77 | 250 |
| IIa6 | | | | 453 | 5.0 | 91 |
| IIa7 | 1110 | 1.74 | | 804 | 3.0 | 268 |
| <i>Examples of formula IIb</i> | | | | | | |
| IIb1 | 80 | 0.04 | 1890 | 20 | 0.07 | 303 |
| IIb2 | 6.0 | 0.007 | 762 | 4.3 | 0.02 | 227 |
| IIb3 | 5.2 | 0.04 | 142 | 3.7 | 0.06 | 66 |
| IIb4 | | | | 9 ^f | 0.29 ^f | 31 ^f |
| IIb5 | | | | 2.9 ^f | 0.25 ^f | 12 ^f |
| IIb6 | 26 | 0.19 | 140 | 11 | 0.21 | 52 |
| IIb7 | 3.1 | 0.03 | 102 | 0.89 | 0.05 | 22 |
| IIb8 | 9.7 | 0.19 | 51 | 4.3 | 0.36 | 13 |
| IIb9 | 3.7 | 0.15 | 25 | 1.44 | 0.24 | 6.3 |
| <i>Examples of formula IIc</i> | | | | | | |
| IIc1 | 196 | 0.55 | 390 | 121 | 1.0 | 120 |
| IIc2 | 150 | 0.21 | 724 | 85 | 0.31 | 271 |
| IIc3 | | | | 425 ^f | 5.1 ^f | 83 ^f |
| IIc4 | 800 | 1.6 | 549 | 392 | 2.6 | 215 |
| IIc5 | 280 | 0.57 | 497 | 209 | 0.85 | 301 |
| IIc6 | | | | 314 ^f | 20 ^f | 16 ^f |
| IIc7 | 1680 | 6.6 | 267 | 856 | 4.3 | 262 |
| IIc8 | 890 | 1.8 | 509 | 214 | 1.5 | 141 |
| IIc9 | 433 | 32 | 14 | 262 | 31 | 8.3 |
| IIc10 | 156 | 11 | 14 | 94 | 11 | 8.2 |

Footnotes for Table 2

^aHuman ovarian: wild-type (NR-) is SKOV3, transfected (NR+) is SC3.2.^bHuman colon: wild-type (NR-) is WIDR, transfected (NR+) is WC14.10.

^cChinese hamster fibroblast: wild-type (NR-) is T-78-1, transfected (NR+) is T79-A3.

^dIC₅₀: the concentration of drug (in micromolar) required to reduce cell numbers to 50% of controls at the end of the evaluation period.

5 ^eRatio = IC₅₀(NR-)/IC₅₀(NR+).

^f4 h exposure.

Table 3. Relative cytotoxicities of selected examples of the compounds of Table 1 in oxic and anoxic tumour cells.

10

| No | IC ₅₀ s in A549 human lung carcinoma cells (4h exposure) in μ M ^a | | | |
|--------------------------------|--|-----------------------|------------------------------|--------------------------|
| | WT ^b anoxic | WT Ratio ^c | P450R ^d anoxic | P450R ratio ^e |
| <i>Examples of formula IIa</i> | | | | |
| IIa5 | 139 | 5.2 | 73 | 9.9 |
| IIa6 | 348 | 2.4 | 31 | 18 |
| <i>Examples of formula IIb</i> | | | | |
| IIb3 | 2.3 | 26 | 0.28 | 133 |
| IIb4 | 4.5 | 7 | 0.45 | 95 |
| IIb5 | 4 | 19 | 0.34 | 150 |
| <i>Examples of formula IIc</i> | | | | |
| IIc3 | 28 | 20 | 2.9 | 146 |
| IIc4 | 82 | 26 | 7.3 | 108 |
| IIc5 | 52 | 30 | 4.5 | 134 |

Footnotes for Table 3. ^aHuman lung carcinoma line.

^bWild-type.

^cRatio = IC₅₀(aerobic)/IC₅₀(anoxic).

15 ^dA549 transfected with human cytochrome P450 reductase (P450R).

It is clear from the data of Tables 2 and 3 that the examples of the nitroaniline derivatives of the invention include compounds which are active as cytotoxic agents, and which have the additional capability of being reductively activated by the *E. coli* NTR and/or by
20 endogenous reductase enzymes under hypoxia.

Wherein the foregoing description reference has been made to reagents, or integers having known equivalents thereof, then those equivalents are herein incorporated as if individually set forth.

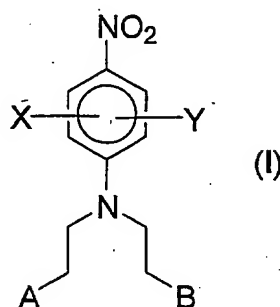
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While this invention has been described with reference to certain embodiments and examples, it is to be appreciated that further modifications and variations may be made to embodiments and examples provided without departing from the scope of the invention.

10

What we claim is

1. A nitroaniline-based unsymmetrical mustard represented by the general formula (I);



wherein X represents one of the groups NO_2 , CN , or SO_2R^1 , where R^1 represents a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R^1 represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

Y represents one of the groups OR^2 , NHCOR^2 , $\text{CONR}^2\text{CO}_2\text{R}^3$, CONR^2 morpholide, CONHR^2 , CONR^2R^3 , CONHOR^2 , $\text{CONHSO}_2\text{R}^2$, SO_2NH_2 , SO_2NHR^2 or $\text{SO}_2\text{NR}^2\text{R}^3$ wherein each R^2 and R^3 independently represent a H, C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

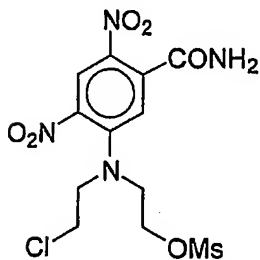
A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

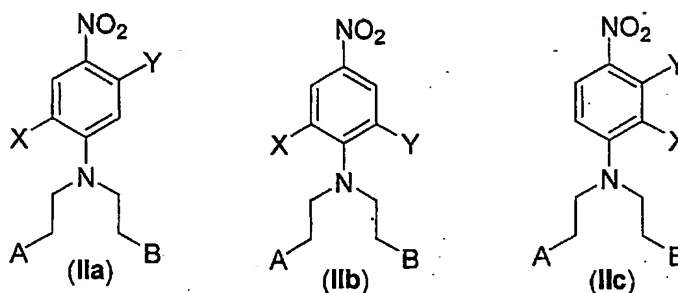
(i) that $\text{A} \neq \text{B}$ and

that



is excluded.

2. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 represented by one of formulae (IIa-IIc)

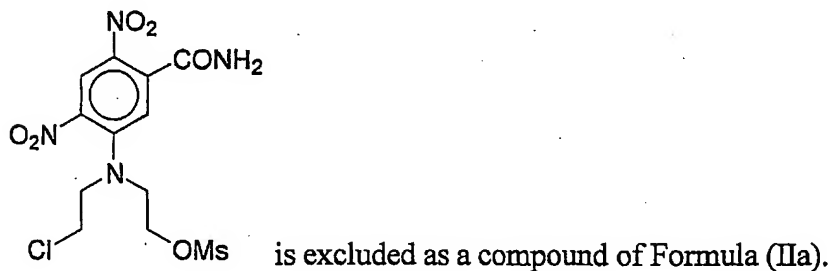


wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

- (i) that $A \neq B$ and

that



3. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 or claim 2 selected from:

5-[(2-Bromoethyl)(2-chloroethyl)amino]-2,4-dinitrobenzamide,

2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,

2-[5-(Aminocarbonyl)(2-iodoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)5-{[(2-hydroxyethyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)5-{[(3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

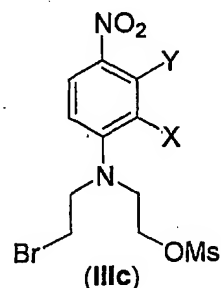
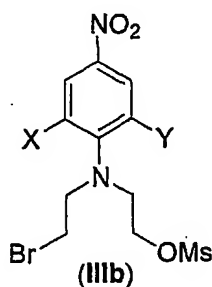
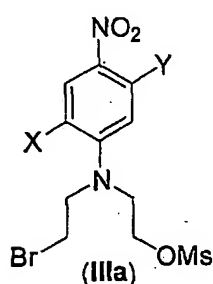
- 2-((2-Bromoethyl)-5-[[2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,
- 2-[2-(Aminocarbonyl)(2-chloroethyl)-4,6-dinitroanilino]ethyl methanesulfonate,
5. 2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,
- 2-((2-Bromoethyl)-2-[[2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,
- 2-((2-Iodoethyl)-2-[[2-hydroxyethyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,
- 10 2-((2-Bromoethyl)-2-[[2-hydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,
- 2-((2-Bromoethyl)-2-[[2,3-dihydroxypropyl)amino]carbonyl}-4,6-dinitroanilino)ethyl methanesulfonate,
- 2-[(2-Bromoethyl)-2-([3-(4-morpholinyl)propyl)amino]carbonyl]-4,6-dinitroanilino]ethyl methanesulfonate,
- 15 Methyl 3-{[2-((2-chloroethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,
- Methyl 3-{[2-((2-bromoethyl){2-[(methylsulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,
- 20 2-[3-(Aminocarbonyl)(2-chloroethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
- 2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,
- 2-((2-Bromoethyl)-3-[[2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,
- 2-((2-Chloroethyl)-3-[[3-hydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,
- 25 2-((2-Bromoethyl)-3-[[3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,
- 2-((2-Bromoethyl)-3-[[4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,
- 30 2-((2-Chloroethyl)-3-[[2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,
- 2-((2-Bromoethyl)-3-[[2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate,

2-[(2-Chloroethyl)-3-({[3-(4-morpholinyl)propyl]amino} carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate and

2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino} carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate.

5

4. The nitroaniline-based unsymmetrical mustard as claimed in claim 1 or claim 2 selected from a compound represented by one of formulae (IIIa-IIIc)



10

wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof.

15

5. The nitroaniline-based unsymmetrical mustard as claimed in claim 4 selected from
2-[5-(Aminocarbonyl)(2-bromoethyl)-2,4-dinitroanilino]ethyl methanesulfonate,
2-((2-Bromoethyl)5-{{(2-hydroxyethyl)amino} carbonyl}-2,4-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)5-{{(3-hydroxypropyl)amino} carbonyl}-2,4-dinitroanilino)ethyl
methanesulfonate,

20

2-((2-Bromoethyl)-5-{{(2,3-dihydroxypropyl)amino} carbonyl}-2,4-
dinitroanilino)ethyl methanesulfonate,

2-[2-(Aminocarbonyl)(2-bromoethyl)-4,6-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)-2-{{(2-hydroxyethyl)amino} carbonyl}-4,6-dinitroanilino)ethyl
methanesulfonate;

25

2-((2-Bromoethyl)-2-{{(2-hydroxypropyl)amino} carbonyl}-4,6-dinitroanilino)ethyl
methanesulfonate,

2-((2-Bromoethyl)-2-{{(2,3-dihydroxypropyl)amino} carbonyl}-4,6-
dinitroanilino)ethyl methanesulfonate,

2-[(2-Bromoethyl)-2-({[3-(4-morpholinyl)propyl]amino}carbonyl)-4,6-dinitroanilino]ethyl methanesulfonate,

Methyl 3-{[2-((2-bromoethyl){2-[(methanesulfonyl)oxy]ethyl}amino)-3,5-dinitrobenzoyl]amino}propanoate,

5 2-[3-(Aminocarbonyl)(2-bromoethyl)-2,6-dinitroanilino]ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{[(2-hydroxyethyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

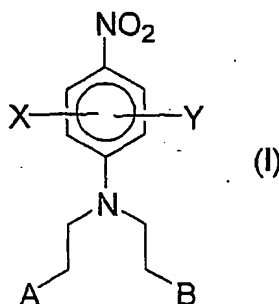
2-((2-Bromoethyl)-3-{[(3-hydroxypropyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

10 2-((2-Bromoethyl)-3-{[(4-hydroxybutyl)amino]carbonyl}-2,6-dinitroanilino)ethyl methanesulfonate,

2-((2-Bromoethyl)-3-{[(2,3-dihydroxypropyl)amino]carbonyl}-2,4-dinitroanilino)ethyl methanesulfonate and

15 2-[(2-Bromoethyl)-3-({[3-(4-morpholinyl)propyl]amino}carbonyl)-2,4-dinitroanilino]ethyl methanesulfonate.

6. A method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula (I);



20 wherein X represents one of the groups NO₂, CN, or SO₂R¹, where R¹ represents a C₁-6-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when R¹ represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;

25 Y represents one of the groups OR², NHCOR², CONR²CO₂R³, CONR²morpholide, CONHR², CONR²R³, CONHOR², CONHSO₂R², SO₂NH₂, SO₂NHR² or SO₂NR²R³ wherein each R² and R³ independently represent a H, C₁-6-lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups; and

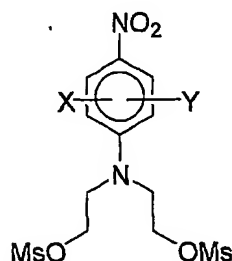
A and B each independently represent halogen, OSO_2R^4 , OSO_2NH_2 , OSO_2NHR^4 or $\text{OSO}_2\text{NR}^4\text{R}^5$, wherein each R^4 and R^5 independently represent a C_{1-6} -lower alkyl optionally substituted with one or more hydroxy and/or one or more amino groups and wherein when each R^4 and R^5 independently represents a tertiary amine the N-oxide derivative of the tertiary amine is further included;
 5 and pharmaceutically acceptable derivatives and salts thereof;
 with the proviso

(i) that $\text{A} \neq \text{B}$

the method including the step of

10

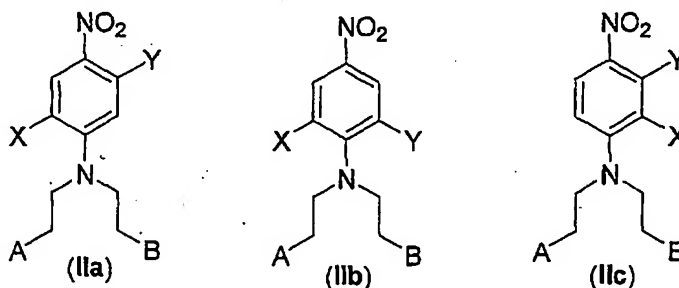
reacting a compound of



with an amount of an alkali metal halide in a polar solvent to give an unsymmetrical halo-mesylate compound.
 15

7. The method of preparing a nitroaniline-based unsymmetrical mustard represented by the general formula represented by one of formulae (IIa-IIc) as claimed in claim 2 or claim 3

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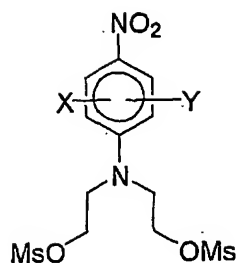


wherein X, Y, A and B are as defined in claim 1 for a compound of Formula (I); and pharmaceutically acceptable derivatives and salts thereof;

with the proviso

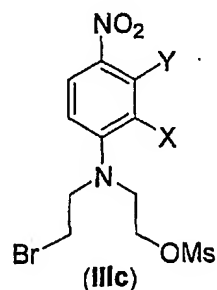
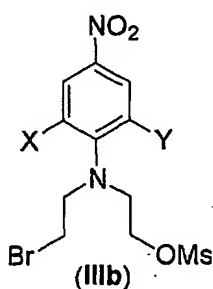
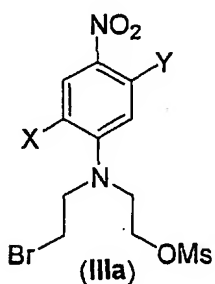
(i) that $A \neq B$ and

the method including the step of
reacting a compound of



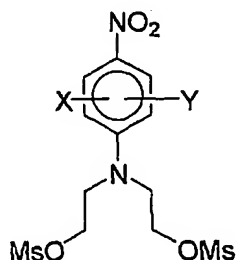
with an amount of an alkali metal halide or mesylate halide in a polar solvent to give
an unsymmetrical halo-mesylate compound.

8. The method of preparing a nitroaniline-based unsymmetrical mustard represented
by one of formulae (IIIa-IIIc) as claimed in claim 4 or claim 5



- wherein X, Y, are as defined in claim 1 for a compound of Formula (I); and
pharmaceutically acceptable derivatives and salts thereof; the method including the
step of

reacting a compound of



with an amount of LiBr in a polar solvent to give a bromo mesylate of one of formulae (IIIa-IIIc).

- 5 9. The method as claimed in any one of claims 6 to 8 wherein the polar solvent is selected from acetonitrile, dimethylformamide, ethyl acetate, triethylamine, acetone and mixtures thereof.
- 10 10. The method as claimed in any one of claims 6 to 9 wherein the alkali metal halide is selected from one or more of the following; LiCl, LiBr, NaI and NaBr.
11. A compound of formula (I) obtained by any one of the methods as claimed in any one of claims 6 to 10.
- 15 12. A method including the step of administering a compound of Formula I as defined in any one of claims 1 to 5 in a therapeutically effective amount to tumour cells in a subject for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as a hypoxia-selective cytotoxin.
- 20 13. The method according to claim 12 wherein the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E.Coli* or by *Clostridia* species.
- 25 14. A method including the step of administering a compound of Formula I as defined in claim 1 in a therapeutically effective amount to target tumour cells in a subject for the use as prodrugs suitable for GDEPT (gene-dependent enzyme-prodrug therapy) in conjunction with at least one nitroreductase enzyme, as an anticancer agent.
- 30 15. The method according to claim 14 wherein the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E.Coli* or by *Clostridia* species.

16. A method of cell ablation therapy utilising at least one nitroreductase enzyme, wherein the method includes the step of administering a compound of Formula I as claimed in claim 1 in a "therapeutically effective amount" to ablate tumour cells in tissue in a subject, wherein said tissue expresses at least one nitroreductase enzyme.
17. The method according to claim 16 wherein the nitroreductase enzyme is encoded for by the *nfsB* gene of either *E.Coli* or by *Clostridia* species.
18. The method according to claim 16 or claim 17 wherein the cell ablation therapy provides a substantially minimal bystander effect.
19. A pharmaceutical composition including a therapeutically effective amount of a compound of formula I as defined in claim 1 and a pharmaceutically acceptable excipient, adjuvant, carrier, buffer or stabiliser.
20. The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in GDEPT to target cancer cells in a subject in need thereof.
21. The use in the manufacture of a medicament of an effective amount of a compound of Formula I as defined in claim 1 for use in cell ablation therapy to target cancer cells in a subject in need thereof.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ03/00225

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. ⁷: C07C 237/30, 309/66, 311/39; C07D 263/04, 295/13; A61K 31/18, 31/166, 31/357, 31/5375; A61P 35/00.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See electronic database consulted below.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN (File CA): substructure search. STN (File CA, Medline, WPIDS): Keywords- prodrug, gdept, mustard, amino?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| P, X | STN File CA, abstract number 138:331354 & N. A. Helsby et al., Chemical Research in Toxicology (2003), 16(4), 469-478. See abstract and CAS Registry Number 517916-68-6 | 1-21 |
| X | F. Friedlos et al., Mustard Prodrugs for Activation by <i>Escherichia coli</i> Nitroreductase in Gene-Directed Enzyme Prodrug Therapy, J. Med. Chem. (1997), 40, 1270-1275. See the whole document, in particular the abstract and page 1272, table 1. | 1-21 |



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
27 November 2003

Date of mailing of the international search report
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